To determine the clinical and laboratory risk predictors of paradoxical reaction in a cohort of patients with Tuberculous lymphadenitis.

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A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF M.D. GENERAL MEDICINE BRANCH I EXAMINATION OF THE TAMIL NADU DR. M.G.R. UNIVERSITY, CHENNAI TO BE HELD IN MAY, 2018

CERTIFICATION

This is to certify that the dissertation **"To determine the clinical and laboratory risk predictors of paradoxical reaction in a cohort of patients with Tuberculous lymphadenitis"** is a bonafide work of Dr.Akhil R carried out under our guidance towards the M.D. Branch I (General Medicine) Examination of the Tamil Nadu Dr. M.G.R. University, Chennai to be held in May, 2018

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Akhil R

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INTRODUCTION Tuberculosis remains one of the deadliest communicable diseases of the world with a worldwide estimate of 10.4 million new TB cases. Tuberculosis is regarded as one of the major health emergencies and is a national priority. In the year 2015 in the south east Asian region, number of deaths due to tuberculosis was 1945 per day (equivalent to 9 passenger planes crashing every day) (1). Tuberculous lymphadenitis is the most common manifestation of extrapulmonary tuberculosis. Despite increasing interest in the recent years and research advances many immunological aspects of tuberculous lymphadenitis remain largely unknown.

The phenomenon of "paradoxical worsening" in which existing lesions worsen or new lesions appear was first described by Choremis in 1955 in children with tuberculosis who developed transient exacerbation of fever and x-ray changes after initiation of antitubercular therapy (2). In fact the first paradoxical reaction may have been observed by Robert Koch when he tried to inject large amounts of killed tubercle bacilli in an attempt to treat patients with tuberculosis, unsuccessfully, which he termed it as "tuberculin shock" (3). It is unpredictable in its timing, severity and duration. A formal case definition for Paradoxical reaction (PR) has not been made, but a consensus definition for use in clinical and research settings has been validated from previous prospective studies. This phenomenon requires worsening of symptoms at the same site or at an anatomically distant site in the absence of Multidrug resistant (MDR) tuberculosis, poor compliance, impaired digestive absorption, absence of another explanation or evidence of treatment failure after an initial improvement on antitubercular therapy is noted (4).

Contents

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tuberculosis, poor compliance, impaired digestive absorption, absence of another explanation or evidence of treatment failure after an initial improvement on antitubercular therapy is noted(4).

This study was designed to define the clinical profile of patients with tuberculous lymphadenitis developing paradoxical worsening and to identify possible clinical and laboratory predictors for the same.

AIMS

To determine the proportion of Paradoxical reaction in a cohort of patients with tuberculous lymphadenitis presenting to a tertiary centre in South India and also to identify clinical and laboratory correlates to predict the same.

OBJECTIVES

1. To determine the proportion of patients with tuberculous lymphadenitis developing paradoxical worsening
2. To describe the clinical profile of patients with tuberculous lymphadenitis developing paradoxical worsening
3. To identify possible clinical and laboratory risk factors for development of paradoxical reactions in a cohort of patients with tuberculous lymphadenitis.

REVIEW OF LITREATURE

Paradoxical reaction is an exuberant inflammatory reaction characterized by worsening of clinical or radiological findings after initiation of appropriate antitubercular therapy in the absence of evidence of drug resistance or presence of an alternative diagnosis(5).

Paradoxical worsening in Tuberculosis is a well-known phenomenon seen in HIV positive patients after initiation of Anti-retroviral therapy. Case definitions for the same in the setting of PLHIV is well described in literature as follows:

(A) Antecedent requirements

Both of the two following requirements must be met:

- Diagnosis of tuberculosis: the tuberculosis diagnosis was made before starting ART and this should fulfil WHO criteria for diagnosis of smear-positive pulmonary tuberculosis, smear-negative pulmonary tuberculosis, or extrapulmonary tuberculosis[44]
- Initial response to tuberculosis treatment: the patient's condition should have stabilised or improved on appropriate tuberculosis treatment before ART initiation—eg, cessation of night sweats, fevers, cough, weight loss. (Note: this does not apply to patients starting ART within 2 weeks of starting tuberculosis treatment since insufficient time may have elapsed for a clinical response to be reported)

(B) Clinical criteria

The onset of tuberculosis-associated IRIS manifestations should be within 3 months of ART initiation, reinitiation, or regimen change because of treatment failure.

Of the following, at least one major criterion or two minor clinical criteria are required:

Major criteria

- New or enlarging lymph nodes, cold abscesses, or other focal tissue involvement—eg, tuberculous arthritis
- New or worsening radiological features of tuberculosis (found by chest radiography, abdominal ultrasonography, CT, or MRI)
- New or worsening CNS tuberculosis (meningitis or focal neurological deficit—eg, caused by tuberculoma)
- New or worsening serositis (pleural effusion, ascites, or pericardial effusion)

Minor criteria

- New or worsening constitutional symptoms such as fever, night sweats, or weight loss
- New or worsening respiratory symptoms such as cough, dyspnoea, or stridor
- New or worsening abdominal pain accompanied by peritonitis, hepatomegaly, splenomegaly, or abdominal adenopathy

(C) Alternative explanations for clinical deterioration must be excluded if possible*

- Failure of tuberculosis treatment because of tuberculosis drug resistance
- Poor adherence to tuberculosis treatment
- Another opportunistic infection or neoplasm (it is particularly important to exclude an alternative diagnosis in patients with smear-negative pulmonary tuberculosis and extrapulmonary tuberculosis where the initial tuberculosis diagnosis has not been microbiologically confirmed)
- Drug toxicity or reaction

ART=antiretroviral therapy. IRIS=immune reconstitution inflammatory syndrome. *It might be difficult or impossible in resource-poor settings to confirm tuberculosis drug resistance and to exclude certain other infections or neoplasia. Cases where alternative diagnoses cannot be fully excluded because of limited diagnostic capacity should be regarded as "probable paradoxical tuberculosis-associated IRIS". In these probable cases, should resolution of clinical or radiological findings of the suspected IRIS episode occur without a change in tuberculosis treatment or ART having been made, they could then be reclassified as "paradoxical tuberculosis-associated IRIS" cases.

Adapted from Tuberculosis-associated immune reconstitution inflammatory syndrome: case definitions for use in resource-limited settings(6)

A formal case definition for paradoxical reaction in non-HIV patients has not been made, however a previous study did put forth a criteria which could be adapted to most settings however this has not been further validated through large scale trials(7).

Table 1: DEFINITION OF PARADOXICAL WORSENING –adapted from Geri et al. (7)

	Paradoxical worsening – Definition
1.	Initial improvement after initiation of antitubercular therapy
2.	Worsening of symptoms at the same site after initial improvement or onset of new TB symptoms or signs at a site anatomically distant from previous after initiation of antitubercular therapy
3.	Absence of MDR tuberculosis, poor compliance, impaired digestive absorption
4.	Absence of any other explanation for the deterioration

PREVALENCE

Paradoxical reaction, even though is not well understood, has been described in literature. Its prevalence is varied across literature ranging from 5 – 25 % and seems to depend on the site of involvement. Cheng et al, found a prevalence of only 2.4% paradoxical worsening in pulmonary tuberculosis whereas its prevalence in lymph node tuberculosis seems to be higher ranging from 12 – 23 %(7–11).

Table 2: Prevalence of Paradoxical Worsening across literature

	Description of article	Prevalence of PR
Smaoui et al(11)	Descriptive retrospective study of Tuberculous lymphadenitis done in Tunisia on a total of 181 patients	18 (12.1%)
Cheng SL et al(8)	659 patients with pulmonary tuberculosis	16 (2.4%)
Cho et al(10)	300 patients with peripheral lymph node tuberculosis from medical records	54 (23%)
Chahed et al(9)	Retrospective study of 501 patients of cervical lymphadenopathy over 12 years	67 (13.4%)
Hawkeye et al(12)	Retrospective study of 109 patients with tuberculous lymphadenopathy	25(23%)
Geri et al(7)	Single centre retrospective study done a 76 amongst HIV negative patients with Tuberculosis	19(25%)

CLINICAL PRESENTATION OF PARADOXICAL WORSENING

Natural history of tuberculosis depends on the interaction of the mycobacterium tuberculosis and the immune response of the host. The innate immune response contains the infection after exposure in 95% of the cases. When reactivation of tuberculosis occurs, most hosts can control the ensuing inflammation in an appropriate manner. In some cases, an exuberant response is noticed after start of treatment which is termed as Paradoxical Worsening(PR). This phenomenon was better recognized in the setting of patients living with Human Immunodeficiency virus(PLHIV) after initiation of HAART in which setting it is aptly termed immune reconstitution inflammatory syndrome(IRIS). Owing to the similar pathogenesis, IRIS and Paradoxical reaction have similar clinical manifestations(13). Leprosy caused by Mycobacterium Leprae, has characteristic inflammatory reactions, type I and II, which is distinct and well described after initiation of anti Leprae therapy(14). A similar phenomenon can also occur in Mycobacterium tuberculosis given the similarity between the organisms in its microbiologic, histopathological and clinical characteristics.

As the definition states, paradoxical worsening in HIV negative individuals can have two distinct types of presentation: one that of worsening of pre-existing disease (after an initial improvement on antitubercular therapy) and the other being occurrence of disease at another site (which was previously not apparently involved). In the former category patient develops enlargement of nodes, fluctuation, tenderness, abscess and fistula formation. The latter category presents as lymph nodal enlargement at another site or involvement of lung, pericardium, pleura, peritoneum, spine, brain, liver, ovary, psoas

muscle, paravertebral muscle and eye. Campbell and Dyson studied 108 patients with lymph node tuberculosis treated with contemporary appropriate treatment and were noted to have developed paradoxical worsening manifested as follows appearance of new lymph nodes(12%), enlargement of pre-existing lymph nodes(13%) and new onset fluctuation of nodes(11%)(4). Hawkeye et al, did a retrospective analysis of 109 patients with Tuberculous Lymphadenitis (most common site of involvement was cervical) and paradoxical worsening occurred in 27 (23%) patients. Median time to occurrence of paradoxical worsening was 46 days (10 – 405 days) and 17 patients(68%) had enlargement of pre-existing nodes, 9 patients(36%) had development of new nodes and 1 each developed lung consolidation, perforated viscus and pericardial effusion(12). Chahed et al, performed a retrospective study amongst 501 patients diagnosed with peripheral cervical lymph node tuberculosis in a tertiary teaching institute in Tunisia. Paradoxical reaction occurred in 67 patients (13.4%) with a median time to onset of 7 months (4 – 9 months). Of these 44.8% had worsening at the previous lymph node site, new lymph nodes appeared in 32.8% patients, fluctuation and fistula formation occurred in 16.4% and 6% patients(9). These studies provide information regarding the type of clinical presentation described in literature for paradoxical worsening in the form of worsening of pre-existing lymph node, recruitment of new sites and involvement of other organ systems.

Table 3: Time to Paradoxical Worsening

	Description of article	Median time to onset Days (IQR)
Cheng SL et al(8)	659 patients with pulmonary tuberculosis	26 days(3 – 100 days)
Cho et al(10)	300 patients with peripheral lymph node tuberculosis from medical records	8 weeks(4- 14 weeks)
Chahed et al(9)	Retrospective study of 501 patients of cervical lymphadenopathy over 12 years	7 months (4 – 9 months)
Hawkeye et al(12)	Retrospective study of 109 patients with tuberculous lymphadenopathy	46 days(21 – 139 days)
Geri et al(7)	Single centre retrospective study done a 76 amongst HIV negative patients with Tuberculosis	86 days(36 – 125 days)

Usually outcomes in paradoxical worsening tends to be excellent especially in lymph nodal tuberculosis, however that's not the case in miliary tuberculosis and CNS tuberculosis. Patients with miliary tuberculosis can present with ARDS. Patients with

CNS tuberculosis could present with cerebral tuberculomas, hydrocephalus, opticochiasmal tuberculosis and arachnoiditis(15–19). Patients can present with pleural effusion as a manifestation of paradoxical worsening with anecdotal reports of chylothorax secondary to mediastinal adenopathy(20). Cheng et al, identified 122 episodes of paradoxical worsening, of which 101(82.8%) occurred in extrapulmonary tuberculosis. Median duration to occurrence of paradoxical worsening was 60 days. 60 episodes occurred in Central nervous system(21).

PREDICTORS FOR PARADOXICAL WORSENING

Undoubtedly, HIV positive status has the greatest association with the occurrence of paradoxical worsening. An HIV positive patient has a 5 times greater odd of developing paradoxical worsening than in non-HIV patient(22). Risk factors for paradoxical reaction in non-HIV patients have been few and described inconsistently.

With the rising incidence of drug resistant tuberculosis, one of the major differential diagnosis considered in the setting of paradoxical worsening is MDR/XDR Tuberculosis. Although an immunopathological mechanism is elucidated, no reliable and rapid diagnostic test is available for its diagnosis. Paradoxical worsening is a diagnosis of exclusion after ruling out drug resistant tuberculosis, drug noncompliance, poor drug absorption and other alternative diagnoses (Sarcoidosis, Lymphoma, Fungal infections). As mentioned above, paradoxical worsening usually gets misdiagnosed as drug resistant tuberculosis resulting in unnecessary institution of toxic second line anti-tubercular drugs. Hence being able to predict and diagnose paradoxical worsening is of utmost

importance as this phenomenon can be managed with continuation of antitubercular therapy, steroids and anti-inflammatory drugs.

Most of the predictors described in literature are based on the current understanding of the immunopathogenesis of this phenomenon. It is proposed that at the onset of the disease there is a relative immunodeficiency at the biological level which gets corrected with treatment resulting in this immune phenomenon. Predictors which suggest the initial immunodeficient state include positive AFB smear (suggesting heavy bacillary load), low baseline absolute lymphocyte count, anemia and negative tuberculin skin test. This is a direct corollary from Immune Reconstitution Inflammatory Syndrome as described in HIV infected patients on Combined Anti-retroviral Therapy in the early phase of tuberculosis (<2 weeks) when the bacillary load is higher. Other studies have found hypoalbuminemia and elevated baseline monocyte count as a predictor of paradoxical worsening(12,23,24). Similarly it's been seen that patients with CSF smears which were positive for bacilli develop CNS related paradoxical worsening like cerebral tuberculomas, opticochiasmal disease and arachnoiditis(25,26).In contrast to increased predilection for conditions with high bacillary load, this phenomenon is seen more in extrapulmonary disease which is classically paucibacillary(24). In the setting of peripheral lymphadenopathy there is antigenic persistence in these anatomic sites which could explain its occurrence(13).

Paradoxical worsening seems to be more common among patients with lymph node tuberculosis and is the most common extra-pulmonary form. As evidenced by Geri et al, out of the 76 patients with tuberculosis, there were 19 episodes of paradoxical worsening, About 72% of cases, were tuberculous lymphadenitis, with PR seen in 13 (68%) of these cases. Thus it seemed that lymph node tuberculosis commonly resulted in paradoxical worsening(7). Risk factors for development of paradoxical worsening included low absolute lymphocyte count (<1000/mm^3) and anemia(<10.5 g/dl)(7). Cheng et al, similarly identified extrapulmonary involvement at initial diagnosis, lower baseline lymphocyte count and greater surge in lymphocyte counts as independent risk factor for paradoxical worsening(24). In another study done in 2007, lower baseline absolute lymphocyte counts and anemia were again identified as risk factors in addition to low body mass index and hypoalbuminemia(8). Cho et al found younger age, male gender and tenderness at the time of diagnosis as independent risk factor for paradoxical worsening(10).On the other hand, Chahed et al, identified lymph node size of >3cm and presence of extra-lymph nodal tuberculosis as independent risk factors for development of paradoxical worsening(9).

Risk factors seem to be different in patients with other forms of tuberculosis, however it seems to follow a pattern which stems from the basic pathogenesis of the disease - immunorestitution. In a retrospective study done in South-Korea amongst patients with 139 patients with pleural tuberculosis, 32(23%) patients developed paradoxical worsening. Risk factors identified included younger age, high serum albumin, low

proportion of lymphocyte and high proportion of PMN in pleural fluid(23). In another retrospective cohort study done in South Korea amongst 458 patients with isolated pleural tuberculosis, paradoxical worsening occurred in 72 patients(16%) with a mean time of development of 8.8 +/- 6.4 weeks, development of paradoxical worsening was independently associated with proportion of eosinophils and protein concentrations in pleural fluid(27). Prospective and retrospective data were collected on TB patients from four UK centres between 1999 and 2008. Of the 1817 patients with tuberculosis, 82 (4.5%) patients developed paradoxical worsening. Frequency was higher among HIV positive(14.4%) than among HIV negative patients(3.8%). This study showed that HIV positive status is the greatest independent risk factor for development of Paradoxical worsening(OR=5). Other risk factors identified included a positive mycobacterial culture and Nucleic acid amplification technique, lack of non HIV immunosuppression, alcohol consumption, smoking, raised baseline ESR and presence of initial disease at extrathoracic lymph nodes. Lymph node disease and positive TB culture had an increased odds of 64.33 and 6.87 fold respectively(22).

At the time of diagnosis of paradoxical worsening there is a surge in the absolute lymphocyte count, inflammatory markers like Erythrocyte Sedimentation Rate and C-Reactive Protein and a change in the tuberculin anergy status from negative to positive. All these findings suggest immune restitution as the mechanism for this phenomenon.

Table 4: Predictive factors described in literature

Younger age(10,12)
Male sex(10,12)
Lymph nodal tuberculosis(22)
Lymph node size > 3cm(9)
Tenderness of lymph node at the time of diagnosis(10)
Associated extra-lymph nodal tuberculosis(9,24,28)
Lymphopenia at baseline(8)
Greater change in Lymphocyte count(8)
Elevated baseline monocyte count(12)
Anemia(8)
Hypoalbuminemia(8)
Positive TB culture(22)

PATHOGENESIS

A precise pathogenesis model has not been developed for paradoxical worsening. Most of our understanding regarding paradoxical worsening comes from indirect measures like tuberculin sensitivity testing and change in laboratory parameters. At the crux of the

pathogenesis is the phenomenon of immune restitution which comes from the understanding that paradoxical worsening belongs to the spectrum of immune reconstitution inflammatory syndrome(29). Evidence of a change in the immune status of the patient while on antitubercular therapy has been consistently proven. Tuberculin test has been found to be negative in patients who are severely ill or hypoproteinemic with reversal of test status after appropriate nutritional supplementation within 2 weeks(30). Similarly, it has been found that patients who develop paradoxical reaction have a negative tuberculin test due to advanced disease at the initiation of therapy which becomes positive after a few weeks of initiation of therapy(31). All these point towards a possibility of immunorestitution, in the pathogenesis of paradoxical worsening. Other theory is that of hypersensitivity to the mycobacterial antigens released after initiation of anti-tubercular therapy like that of a Type II Lepra reaction. Lipoarabinomannan, a protein from the cell wall of M.Tuberculosis has been implicated in this hypersensitivity reaction by causing the release of TNFα from mononuclear phagocytes. In fact patients who develop paradoxical TB IRIS have higher pretreatment levels of lipoarabinomannan(13).

ROLE OF IMMUNORESTITUTION IN PARADOXICAL WORSENING

T cells can be classified into T Helper cells and T cytotoxic cells. T helper cells can be further classified based upon the profile of cytokines secreted by them into T helper 1 and T helper 2 cells(32). T helper 1 cells secrete Interferon (IFN) – γ and Interleukin (IL) – 2 whereas Th2 cells produce IL-4, IL-5, IL-6, IL-10 and IL-13. T helper 1 cells are responsible for the cell mediated immunity and T helper 2 cells are responsible for

humoral immunity and allergic responses. In addition, the cytokines produced by a T helper subset seems to propagate the production of the same subset and inhibit the production of the other Th subset. Differentiation of T helper cells into each of these subsets depends on the cytokine microenvironment. For instance, the presence of IL-12 secreted by macrophages, results in differentiation of T helper cells into T helper 1 cells and production of IFN-γ. On the contrary presence of IL-4 results in differentiation of T helper cells into T helper 2 cells. The cytokines produced by Th1 subset seems to be pro-inflammatory whereas cytokines produced by Th2 subset seems to be anti-inflammatory(33,35,36). Th1 response even though is pro-inflammatory is microbicidal in contrast to the Th2 response. In the presence of excess of Th2 response, the microbicidal response of Th1 is suppressed as explained above. In active tuberculosis, it has been found that at the outset there is a Th2 predominant response which shifts to a Th1 predominant response. This was clearly established it was found that PPD stimulated PBMCs in active pulmonary TB at the time of diagnosis have depressed production of immunoprotective cytokines – IFNγ and IL-2 which are essential to contain Mycobacterial infection and to form functional granulomas. Instead there is increased production of immunosuppressive and macrophage deactivating cytokines – IL-10 and TGF β(34). This Th2 predominant response is responsible for the tuberculin anergy in some of the patients with active tuberculosis, as evidenced by the increased levels of IL-4 and IL-10 and lower levels of IL-12 positive cells in the subset of patients with negative tuberculin test(31). Undoubtedly, the immune response which predominate at the onset of the disease in active tuberculosis is a Th2 response(35).

As the treatment progresses it has been found that the cytokines expressed by the Th2 response, TGFβ and IL-10, progressively decline(34). At the same time, previously negative tuberculin skin test become positive, in other words the balance of the immune response shifts to the Th1 arm. This would result in release of proinflammatory cytokines resulting in worsening of lymph nodes, lung lesions, occurrence of pleural effusion, tuberculomas, hydrocephalus(36).

This transition from Th2 to Th1 response happens in all patients who have developed active tuberculosis. The reason why a patient with active tuberculosis is unable to contain the infection is due to the Th2 predominant response rather than the Th1 predominant response. Various factors that have been considered to be responsible for this polarization could be the age, host genetics, cytokines in the microenvironment, the type, dose and localization of antigens, type of antigen presenting cells, co-stimulatory molecules(37). Thus, immune recovery is the norm in patients being treated for tuberculosis. The exactness and appropriateness of the immune recovery determines whether the patient develops paradoxical worsening. An overwhelming immunorestitution would result in a more severe paradoxical worsening. Thus, the degree of paradoxical worsening would depend on the initial immunological state of the patient and the overall balance of the pro-inflammatory and anti-inflammatory cytokines.

Even though it is well described that Th2 to Th1 transition occurs in patients with tuberculosis, it has never been conclusively established, to what happens to the Th1 response which the patient has developed during the treatment. Does it persist till the

completion of therapy? Does it change over to an anti-inflammatory Th2 later? Future research must also emphasise on elucidating this part of the pathogenesis.

ROLE OF T Regulatory cells in the Immunopathogenesis of Tuberculosis and Its Role in Paradoxical Worsening

Pathogenesis of Tuberculosis can be described as chronic persistent antigenic stimulation, which maintains a sustained immune response against the bacilli but fails to eradicate the same. This immune response does have a regulatory component to it which has been well elucidated off late. This regulatory function is mediated by a subset of CD4 cells – CD4+CD25+ regulatory cells which inhibits the actions of CD4 and CD8 cells. They were initially described in relation to self-tolerance and prevention of autoimmune conditions. They have been described with chronic infections like tuberculosis too. Specific function of Regulatory T Cells is suppression of specific immune response produced by the T helper 1 cells. This is evidenced by the demonstration of increased expression of IFNγ, a T helper 1 marker, on suppression of regulatory T cell expression(38,39). Thus, suppression of these cells may play a role in the immunopathogenesis of paradoxical response, however there is no conclusive evidence for the same. There has been indirect evidence of its role based on the effect of vitamin D status of the body and occurrence of paradoxical worsening. Vitamin D has been found to have an anti-inflammatory effect by promoting FoxP3 and CTLA4, which are markers of regulatory T cells(40).

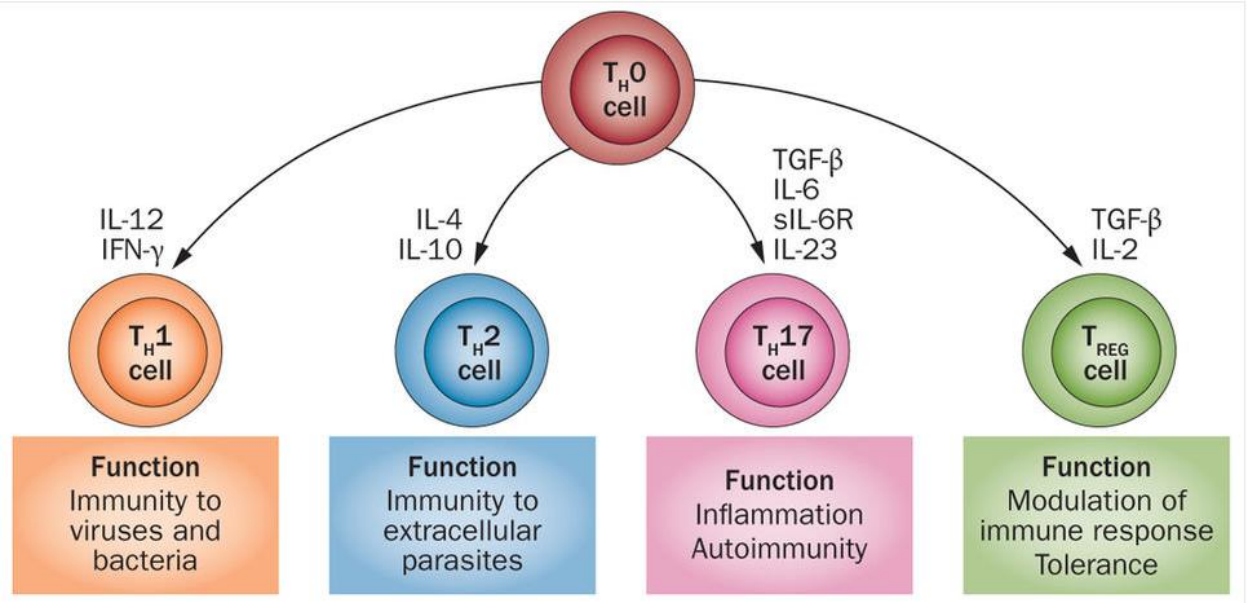


Figure 1: Subdivisions of the T helper response and its respective functions, Adapted from IL-6 biology: implications for clinical targeting in rheumatic disease(41)

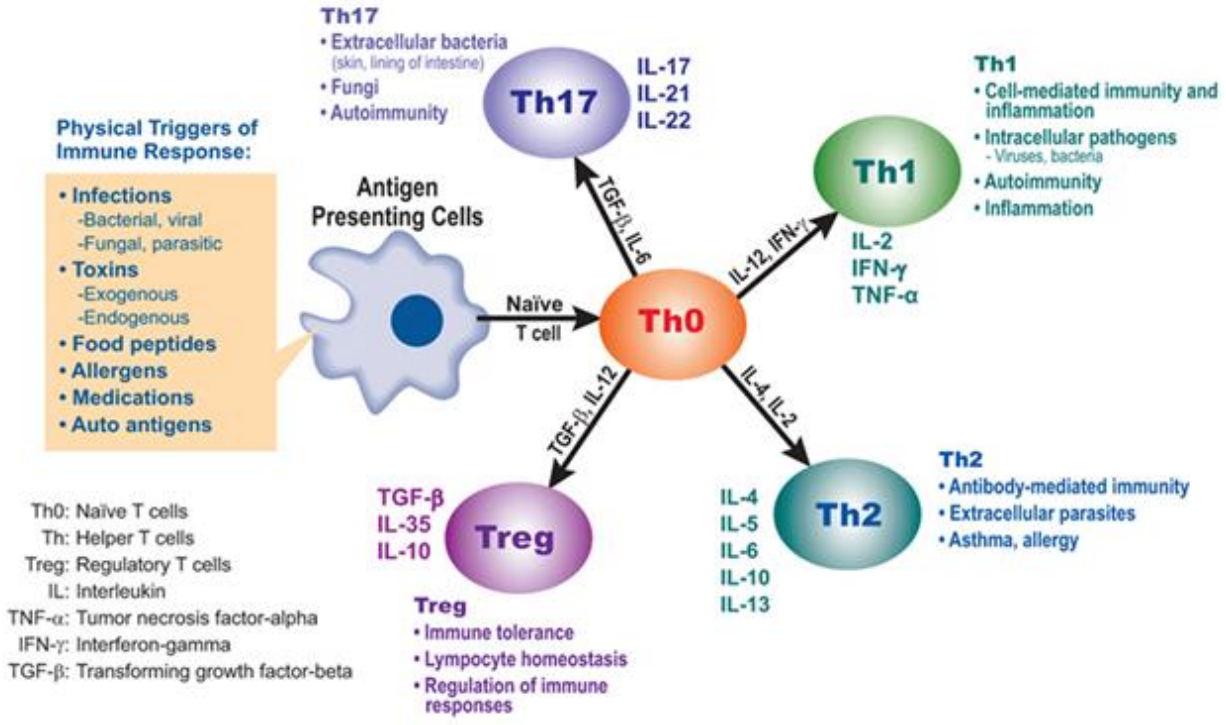


Figure 2: T helper response effector response (adapted from berger et al)(33)

HYPERSENSITIVITY TO MYCOBACTERIAL ANTIGENS

Another important cytokine known to play an important role in the immunopathogenesis of tuberculosis is TNFα. TNFα is secreted by macrophages and monocytes in response to Mycobacterium tuberculosis. It was seen that live mycobacterium was able to trigger the release of TNFα(42). In 1989, lipoarabinomannam, mycobacterial cell wall glycolipid, was reported to stimulate the release of TNFα from human and murine macrophages(43). Another important antigen implicated in the stimulation of TNFα is 30 kDa α antigen(44). The role of TNFα is further elucidated from reports of increased occurrence of paradoxical worsening in patients who develop tuberculosis while on treatment with

TNFα blockers, after stopping TNFα blockers(45,46).TNFα plays a major role in the pathogenesis of tuberculosis:

1) TNFα promotes apoptosis of mycobacteria infected cells

2) TNFα promotes the maturation of dendritic cells which in turn activates the cellular immunity

3) TNFα increases the antimicrobial activity of macrophages

4) TNFα plays a major role in the recruitment of monocytes and antigen specific T lymphocytes(47)

5) TNFα is critical in the formation and maintenance of granuloma complex(48,49)

6)Promotion of central caseation which promotes mycobacterial persistence within the granuloma(50)

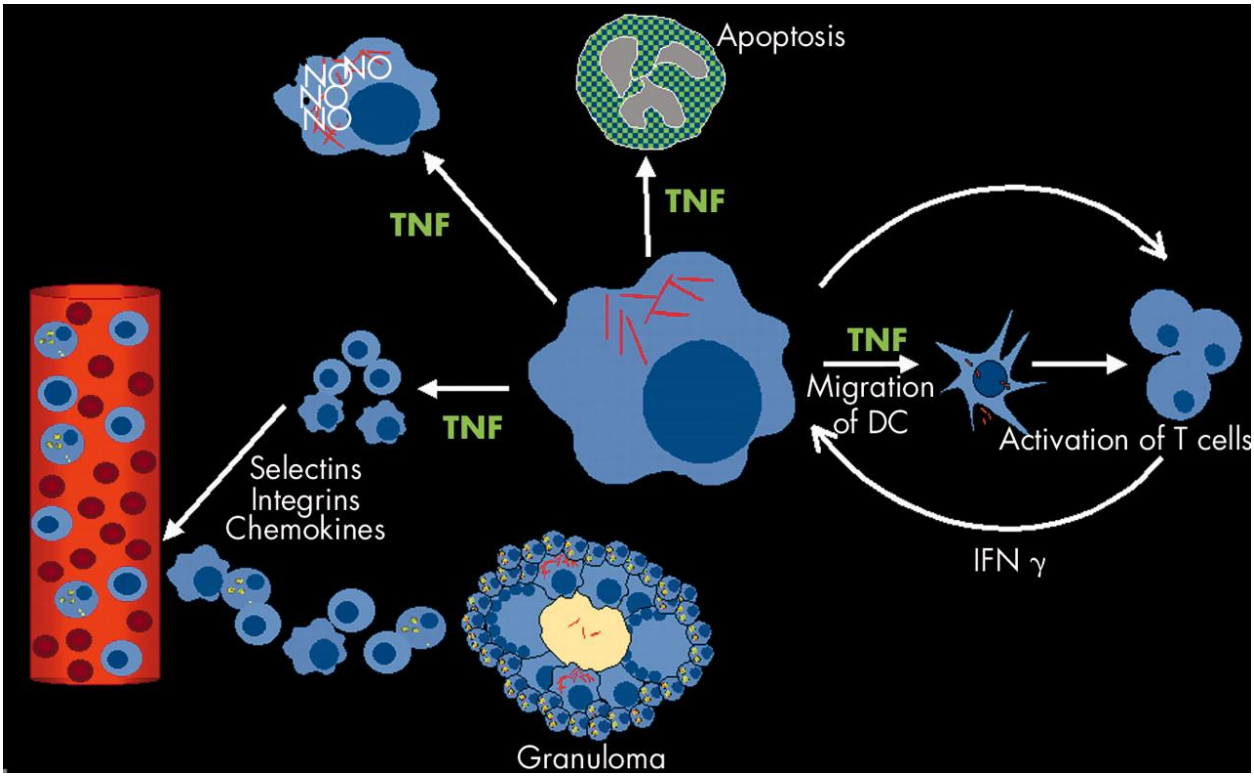


Figure 3: Role of TNFα in the pathogenesis of Tuberculosis, adapted from Immunological control of tuberculosis: role of tumour necrosis factor and more, S Stenger(47)

Thus, TNFα plays a major role in the inflammatory reaction of paradoxical worsening and thus, can be a potential target for treatment as described in the treatment section of this dissertation.

TREATMENT OPTIONS

There are no definite guidelines for treatment of Paradoxical worsening. Treatment options available are based on the understanding that this phenomenon is an exaggerated immune response after initiation of appropriate antitubercular therapy. Hence treatment described in literature aims at curbing this hyperactive immune response. Treatment options available in lymph nodal tuberculosis with paradoxical worsening include: surgical management and medical management. Surgical management could range from

a mere aspiration of pus from the lymph node of concern to extensive debridement of the lymph node and sinuses. This could serve as both a therapeutic measure as well as diagnostic measure to rule out resistance and alternative diagnosis.

Surgical management of Paradoxical Worsening of Tuberculous Lymphadenitis

Traditionally, role of surgery, in a case of tuberculous lymphadenitis is limited to histopathological and microbiological diagnosis. However, in the case of paradoxical worsening it has been found that removal of the site of inflammation, would result in resolution of symptoms. This is applicable for patients who does not respond to the medical treatment or the disease is extensive or patient has extensive discharging sinuses. A retrospective chart review of patients referred for surgical relief of symptom, had 6 patients with discharging sinus and were on antitubercular therapy for at least 3 months. Amongst these 14 patients, 10 underwent lymph node excision, 3 underwent excision along with incision and drainage of cold abscesses and 1 patient underwent incision and drainage with debridement(51).

Table 5: Surgical Management of patients with paradoxical worsening adapted from gaikwad et al(51)

Age (Mean)	26.1 (Range: 15 – 40)
Sex (Male: Female)	1:13
Presentation: a. Swelling b. Sinus c. Constitutional Symptoms d. Disseminated	 a. 14 (100%) b. 6(42.9%) c. 6(42.9%) d. 3(21.3%)
Mean duration of symptoms	6.8 months
Mean duration of Antitubercular Therapy	7.4 (Range: 3 – 15) months
Surgical Procedures performed a. Lymph node excision b. Lymph node excision with Incision and drainage c. Incision and drainage with debridement	 a. 10 b. 3 c. 1
Types of Lymphadenectomy a. >1 lymph node level excised	 a. 5

b. Single Level excised	b. 9

Surgical approach was used in 71.6% of patients with Paradoxical worsening in a retrospective study done in Tunisia(9). Surgical excision of fluctuant lymph nodes, abscesses and sinuses could shorten the duration of antitubercular therapy, give symptomatic relief and help in the healing process.

Medical Management in Paradoxical Worsening

Being an inflammatory reaction, steroids has been used as a treatment option in patients with paradoxical worsening. Use of steroids is further supported by its use in patients with tuberculous meningitis (depending on Medical Research Council Stage) to prevent inflammatory complication post initiation of antitubercular therapy(52). Effectiveness of steroids is not well established with variable outcome in literature from rapid clinical improvement to persistence or worsening(53–56). Use of corticosteroid will help in reducing the edema around the enlarged nodes and can decrease the symptoms due to the mass effect of the enlarged nodes.

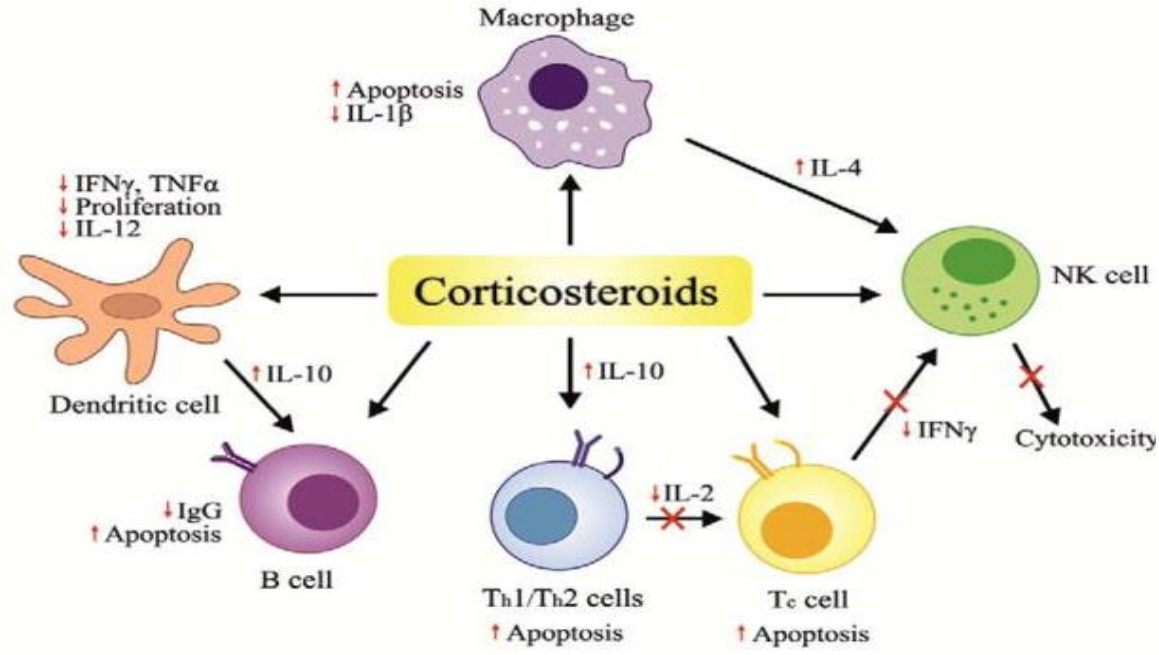


Figure 4: Mechanism of action of corticosteroids in tuberculosis (Adapted from Novel Adjunctive Therapies for the Treatment of Tuberculosis
A.A. Ordonez,[1,2,3,§] M. Maiga,[2,4,§] S. Gupta,[2,4] E.A. Weinstein,[1,2,4] W.R. Bishai,[2,4] and S.K. Jain[1,2,3,*])(57)

In a retrospective study done at Chung Ang Hospital and Yong sang hospital,139 patients with pleural tuberculosis were reviewed, of which 32(23%) developed paradoxical worsening. Amongst these 32 patients, 18 patients needed additional treatment. Seven patients were initiated on steroids at 0.5 mg/kg/day showed improvement in symptoms within a median duration of 14 days. Patients who received steroids seems to have responded quicker than the non-steroid recipients(23). In a retrospective chart review done in Tunisia, amongst the 67 patients who had paradoxical worsening, only 2 received steroids, full dose antitubercular therapy was reinitiated on 7 (10.4%) patients and surgical excision was done in 48 (71.6%) patients. Another descriptive retrospective

study done in Tunisia with 181 patients with Tuberculous Lymphadenitis, 18 patients developed paradoxical worsening. All of them needed steroids with 15 of them showing improvement and 3 having persistent lymph nodes(11).

Table 6: Treatment options described in literature

Study with details	Treatment used	Steroid dosage and other remarks
Hawkeye et al(12) 109 patients with Lymph Node Tuberculosis Paradoxical Reaction – 25 patients (23%)	• Prednisone only – 10 patients (37%) • Aspiration only – 7 patients (26%) • Both – 4 patients (15%) • Neither – 6 patients (22%)	• Mean dose: 60 mg/day • Range: 20 – 90 mg/day • Duration: 52.5 days(mean); (Range 14 – 169 days)
Jung et al(23) 139 patients with Pleural Tuberculosis Paradoxical reaction-	• No additional treatment (14 Patients) • Steroids – 7 patients	• Prednisone – 0.5 mg/kg • Improvement within a median duration of 14 days

32(23%)	• Simple drainage – 7 patients • Thoracotomy – 4 patients	• Rapid recovery in the steroid arm
Chahed et al(9) Retrospective review of 501 patients with lymph node tuberculosis Paradoxical reaction – 67 patients (13.4%)	• Steroids – 2 (3%) • Restarting ATT – 7 (10.4%) • Surgical excision – 48(71.6%) • Ciprofloxacin – 13(19.4%) • Addition of Ethambutol – 9 (13.4%) • Continuation of classic treatment – 2(3%)	• No details about steroid dosage available
Smaoui et al(11) 181 patients with TB Lymphadenitis	• All 18 patients received adjunctive steroids	• 15 patients showed improvement • 3 had persistent lymphadenopathy

Paradoxical reaction – 18 patients (12%)		
Geri et al(7) 76 Patients with tuberculosis with atleast 1 extrapulmonary involvement Paradoxical reaction – 19 (25%)	• Surgical treatment – 11 patients • Steroids – 9 patients • Extension of ATT duration – 4 patients • No specific treatment – 5 patients	• 0.5 mg/kg • Median duration of extension of ATT - 3 months
Cheng et al(21) Review of 122 episodes of paradoxical worsening across literature	• Steroids – 48 (39.3%) • Surgical intervention – 74 (60.7%) • Change in Antitubercular Regime – 19(15.6%) • Conservative management – 17 (13.9%)	• 95 episodes resolved with complete recovery

In a retrospective study done in Paris between 2000 and 2010, consisting of 76 patients with tuberculosis with at least 1 extrapulmonary involvement, paradoxical worsening occurred in 19 patients, of which 9 patients received steroids, 4 patients were just continued antitubercular therapy for an extended period, 11 patients needed surgical drainage and 5 patients did not receive additional treatment. Another interesting finding in this study was while treatment for paradoxical worsening was going on paradoxical worsening recurred in 4 patients at least once. Two of them needed surgical treatment and 3 needed oral steroids(7). Chen et al, reviewed 122 episodes of paradoxical worsening, of which about two-thirds patients needed surgical treatment, close to 40% needed steroids and more than two-thirds recovered with no residual disease(21).

Long term use of steroids would be associated with steroid induced diabetes mellitus, hypertension, and weight gain. TNF-α plays a major role in the granuloma formation and in the inflammatory reaction in patients with paradoxical worsening to the mycobacterial antigens, as explained in the pathogenesis section of this dissertation. TNF-α blockade, seems to be other options in patients with recalcitrant paradoxical worsening. Options of TNF-α blockers include, anti TNF blockers like infliximab, adalimumab, etanercept; other TNF blockers like Thalidomide which seems to reduce leukocytosis, brain morphology and the symptoms in rabbit models and Pentoxiphylline(57). There are case reports, showing the beneficial effects of use of Infliximab in curbing the inflammatory response in neurotuberculosis(58). Thus, this could also be a therapeutic option, even

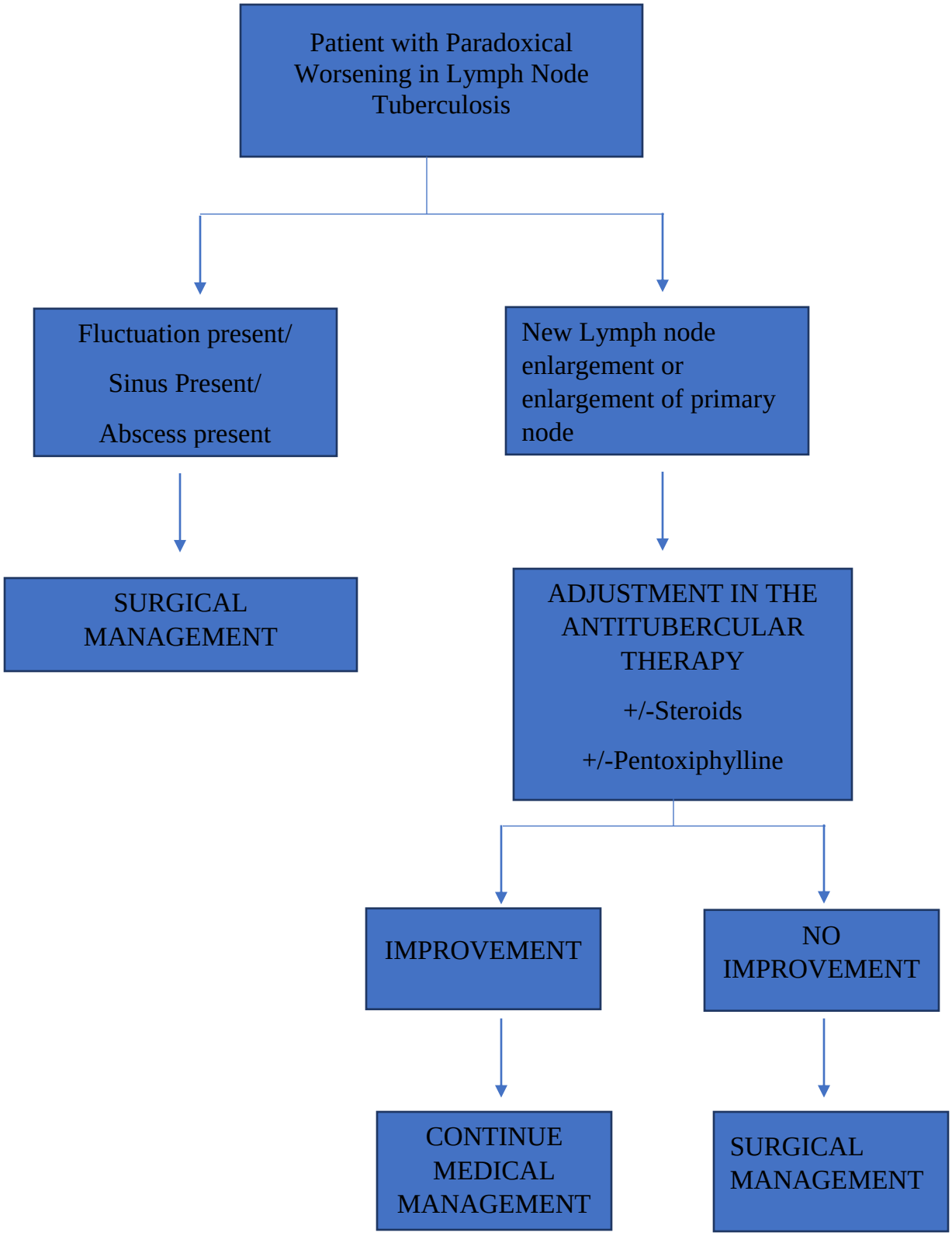
though there are not many studies to substantiate the same, which is important, especially with the known fact that, TNF-α blockers are known to reactivate latent tuberculosis.

Other options described are Phosphodiesterase inhibitors, which increase intracellular cyclic AMP levels which in turn decrease the TNF-α levels. Animal models of tuberculosis have shown beneficial effects with the adjunctive use of PDE-I which include Theophylline, Cilostazol, Sildenafil, Tadalafil and Rofilumast(57,59,60).

Thus, treatment of Paradoxical worsening can be summarized as:

1. Medical Management
    a. Conservative Management – wait and watch
    b. Restarting/continuing Quadruple Antitubercular Therapy
    c. Corticosteroids – 0.5 mg/kg – 1 mg/kg, short course
    d. Newer options: (Not proven)
        i. TNF-α blockers – Pentoxiphylline, Thalidomide
        ii. Specific TNF-α blockers – Infliximab, Etanercept, Adalimumab
        iii. Phosphodiesterase inhibitors – Theophyline, Cilostazol, Sildenafil, Tadalafil and Rofilumast
2. Surgical Management
    a. Incision and drainage of abscess
    b. Lymph node excision
    c. Lymph node excision with debridement

Figure 5: Treatment algorithm proposed, adapted from chahed et al(9)



MATERIALS &METHODS

Setting

This study was carried out in Christian Medical College (CMC), Vellore, which is a 2700-bedded tertiary care teaching hospital in South India. The hospital serves the population of Tamil Nadu and the neighboring state of Andhra Pradesh, besides being a referral center for patients from other parts of the country and the Indian subcontinent.

Patients were recruited from May 2016 through June 2017 and were followed up till 6 months after recruitment. Patients who were recruited in the month of May 2017 and June 2017 were followed up till the completion of intensive phase.

Study Design

This was a prospective cohort study in which patients with tuberculous lymphadenitis(TBLN) initiated on antitubercular therapy(ATT) were followed up to look for development of paradoxical worsening and identify possible risk factors for its development

Participants

A consecutive sampling strategy was employed for this study, wherein all patients above the age of 15 years, who presented with tuberculous lymphadenitis to outpatient and inpatient departments of Infectious Disease, Medicine and Surgery, at CMC Vellore, were enrolled into this study.

Inclusion criteria:

Patients > 15 years with peripheral Tuberculous lymphadenitis:

a. who were planned for initiation of antitubercular therapy or
b. Who were already on antitubercular therapy for less than 30 days and presented to us for reconfirmation of diagnosis

Exclusion criteria:

a. Isolated Mediastinal or abdominal lymph nodal involvement with tuberculosis
b. Human Immunodeficiency Virus(HIV) infection
c. Multidrug resistant tuberculosis(MDR) or Extensively drug resistant tuberculosis(XDR) as identified on tissue sample by molecular or culture techniques
d. Patients with connective tissue disorder or malignancies or immunodeficiency
e. Patients who have been on steroids and immunosuppressants

Study Procedure:

All patients with confirmed or probable tuberculous lymphadenitis who were to be initiated on antitubercular therapy presenting to Outpatient and Inpatient Department of Infectious Disease, General Surgery and General Medicine were approached for participation in the study. A written informed consent was obtained from those eligible and willing to participate. In case of patients less than 18 years, consent was taken from the guardian after explaining the nature of the study to the patient.

Case definitions:

Confirmed Tuberculous Lymphadenitis(TBLN): Patients were considered as confirmed tuberculous Lymphadenitis when the patient had characteristic clinical findings, histopathological findings of tuberculous lymphadenitis with PCR/culture positivity for Mycobacterium tuberculosis in the tissue sample.

Probable Tuberculous Lymphadenitis: Patients were considered as probable tuberculous Lymphadenitis when clinical and histopathological findings were characteristic of tuberculous lymphadenitis with negative microbiology and positive response to therapy.

Paradoxical Reaction/ Worsening (PR): Patients with tuberculous lymphadenitis was diagnosed to have Paradoxical worsening if the patient fulfilled the following diagnostic criteria which was adapted from previous studies on paradoxical worsening:

A. Initial improvement after initiation of antitubercular therapy
B. Worsening of symptoms:
    a. At the primary site: increase in the size of the lymph node, development of tenderness, fluctuation and sinus formation
    b. Development of new nodes with or without signs of inflammation at a site anatomically distant from the previous site
C. Absence of MDR tuberculosis, poor compliance, impaired digestive absorption
D. Absence of any other explanation for the deterioration

Being a referral centre and from our experience we had found that there was this distinct group of patients who were on antitubercular therapy from elsewhere and presented to our centre with paradoxical worsening assuming drug resistance or clinical failure. If we were to exclude these patients we were likely to miss valuable information characterizing this distinct cohort of patients. Hence, we designed the study to incorporate these patients, thus having two arms of recruitment.

Patient recruitment and assessment:

Incident cohort:The first cohort included patients diagnosed with TB lymphadenitis for the first time at our centre. This also included patients who were diagnosed elsewhere but were on ATT for less than 30 days. These patients would not be having paradoxical reaction at presentation.

Paradoxical Reaction/Worsening (PR) cohort:The second cohort would include all patients who presented with paradoxical worsening at first presentation to our centre.

This design incorporating two arms was done to facilitate obtaining a possible incidence of development of paradoxical worsening as well as define this PR group better in view of the low incidence in previous literature.

BASELINE ASSESSMENT FOR THE INCIDENT COHORT:

At baseline, lymph node groups, which were involved were identified by clinical examination. Both clinical and laboratory parameters were assessed for these patients at baseline which were as follows:

Clinical parameters

a. Size of the lymph node
b. Site of lymph node
c. Number of lymph nodes
d. Presence of local tenderness
e. Presence of fluctuation
f. Presence of discharge

Assessment of the size of lymph node

To avoid interobserver variation in the measurement of lymph nodal size, a standardized technique was used for the measurement of lymph nodal size between patients and also for the same patient during the course of treatment. Following steps were followed:

1. Vernier calliper was used for all measurements of lymph node
2. The lymph node in question was held with two fingers
3. The outline of the lymph node was drawn
4. Largest diameter was measured

5. Diameter of a line which is perpendicular to the largest diameter was then measured
6. These measurements were done 3 times and an average was taken
7. Photograph of the lymph node were taken and stored for future purpose

These steps were followed at each measurement subsequently at follow-up.

Laboratory parameters:

Laboratory parameters assessed at baseline included:

a. Hemoglobin
b. Erythrocyte Sedimentation Rate(ESR)
c. C- Reactive Protein
d. Total WBC count, Differential Count, Absolute Lymphocyte count(calculated)
e. CD4 count.

Details regarding patient demographics, clinical findings – lymph node size, characteristics and above test results were entered in a data abstraction sheet designed for this study. Subsequent patient evaluation and management was entirely at the discretion of the treating physician.

FOLLOW-UP OF THE INCIDENT COHORT:

The study participants were followed-up at the end of intensive phase (2 months) and then at end of treatment which varied from 6-9 months. At each follow-up visit,

following clinical and laboratory parameters were assessed, to check if they had developed a PR:

Clinical Parameters

a. At the primary site of involvement:
    a. Size of lymph node: any increase or decrease in size of lymph node
    b. Number of the lymph node: any increase in the number of lymph node
    c. Presence of local tenderness (newly detected)
    d. Presence of fluctuation (newly detected)
    e. Presence of discharge (newly detected)
b. At other sites:
    a. Any new nodes which were not present in the previous visit
    b. Assessment for signs of inflammation

Laboratory parameters

Following laboratory parameters were assessed at each follow-up visit:

a. Hemoglobin
b. ESR
c. CRP
d. Absolute Lymphocyte count (ALC)

PR COHORT AT FIRST VISIT

These patients were diagnosed to have Paradoxical worsening based on history and clinical presentation as reported by patients and fulfilling the criteria as per our definition. Similar clinical and laboratory parameters were assessed for this group as well.

At the time of diagnosis of paradoxical reaction in the incident cohort and the PR cohort, apart from the aforementioned parameters, poor drug compliance and drug resistance were also assessed. We performed a pill count, followed up previous cultures to rule out resistance (Incident cohort) and if there was a doubt of the same, repeat biopsy was advised to the patient for confirmation of the diagnosis and performed if the patient consented for the same. In case of lymph node abscess if surgical aspiration/debridement/drainage was planned for therapeutic purposes, confirmation was done based on pus mycobacterial PCR and/or culture positivity.

Outcomes assessed

PRIMARY OUTCOME:

To determine the proportion of patients diagnosed as tuberculous lymphadenitis developing Paradoxical worsening.

SECONDARY OUTCOME:

To elaborate the clinical profile of patients developing Paradoxical worsening

To identify baseline clinical and laboratory parameters, which could predict the occurrence of paradoxical worsening in this cohort

Sample Size

We utilized the observational study done by Dr Vincent Cheng to calculate the sample size for the study. In his observational study, the incidence of paradoxical reaction ranges from 10-15%. Implementing these values,

$$N = 4pq/d^2$$

$p = 0.10$, $q = 1-0.10= 0.90$

$d = 0.05$

$N = 138$

Statistical Analysis:

Data entry was done using EPIINFO Software. Frequencies in the 2 groups – Paradoxical Reaction versus non-Paradoxical Reaction were estimated and univariate analysis was done using chi-square test for dichotomous variables and a students' T test for continuous variables.

Institutional review board and ethics committee clearance:

The study design and methods were approved by the institutional review board (blue) and ethics committee of Christian Medical College, Vellore (IRB Min. No. 10021 dated 04.04.2016). A copy of the IRB approval statement can be found in Annexure 1.

Funding of the study:

This study was funded by fluid research grant number 22Y909 of Christian Medical College, Vellore.

As per the study design, we recruited consecutive patients with tuberculous lymphadenitis presenting to Infectious Diseases, General Medicine and surgery outpatient and inpatient departments. Patients who were later found to have an alternative diagnosis, Multidrug resistant and Extensively Drug Resistant Tuberculosis were excluded from the study

Study Flow Diagram:

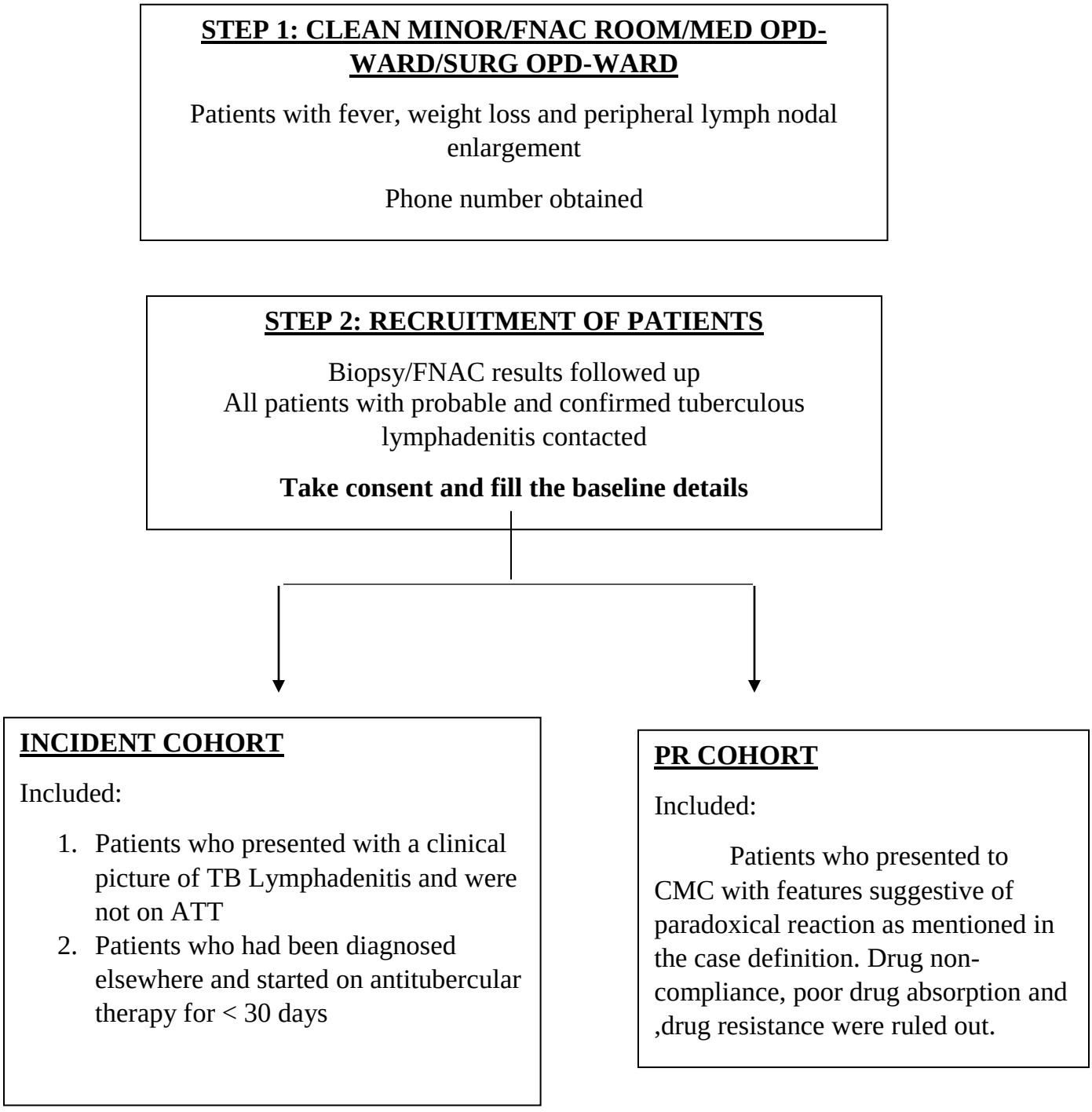


FIGURE 6: Study flow diagram

STEP 3: BASELINE CLINICAL AND LABORATORY ASSESSMENT

Clinical assessment: Lymph node size, site, number, fluctuation, tenderness or sinus
ESR/CRP
ALC/CD4
Weight based ATT initiation by the treating physician

STEP 4: ASSESSMENT AT PR(EVENT), END OF INTENSIVE PHASE AND CONTINUATION PHASE

Clinical assessment: Lymph nodal size, fluctuation, tenderness and sinus

ESR/CRP
ALC

RESULTS

This prospective cohort study was done from May 2016 through June 2017. During this period 80 patients were recruited. Four patients were excluded, as one patient refused to provide consent, one patient had an alternative diagnosis and the other 2 had multidrug resistant tuberculosis. Thus, seventy-six (N= 76) patients were included in the final analysis (Figure 7).

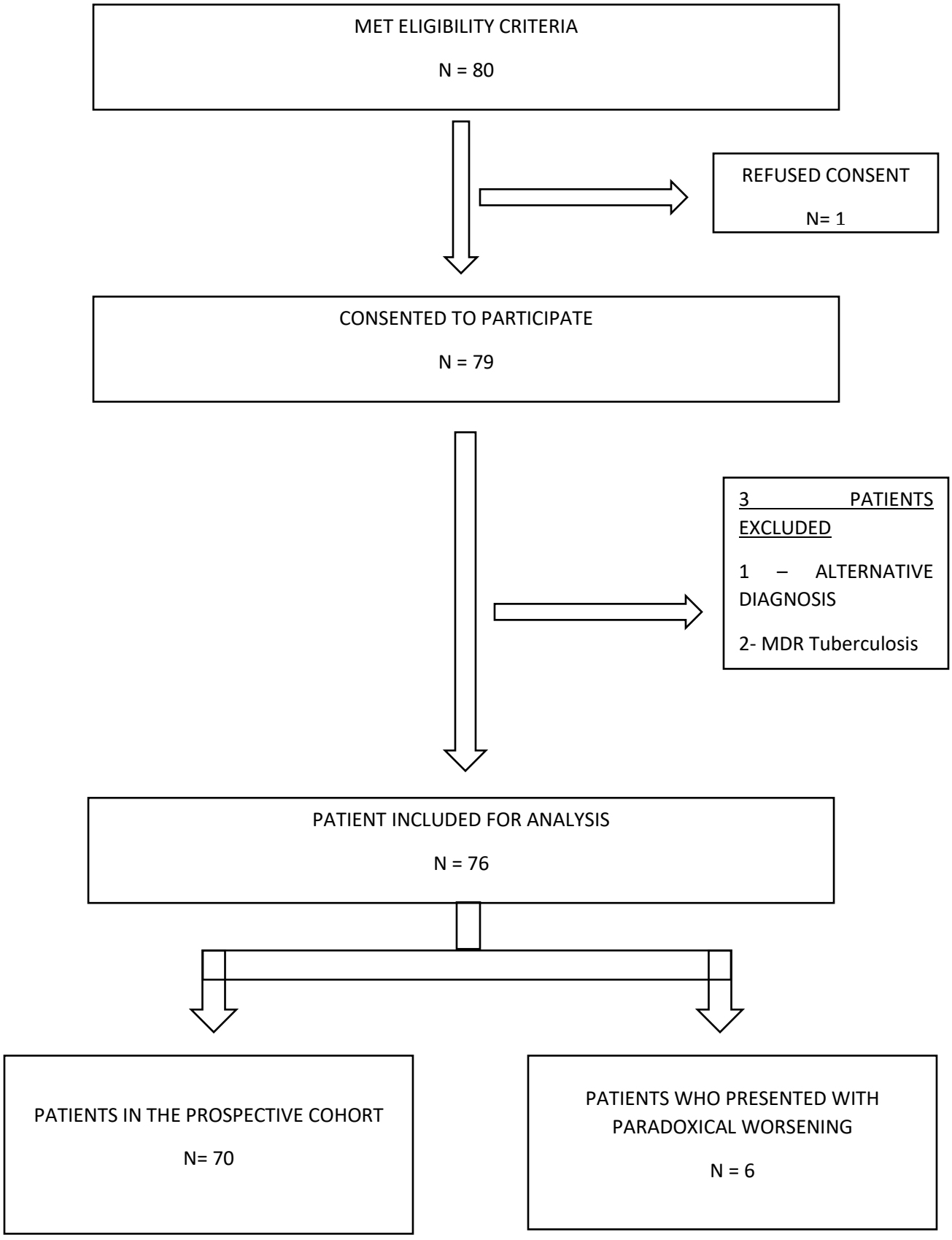


Figure (7) STROBE Diagram

INCIDENT COHORT

Baseline characteristics of the incident cohort (N = 70)

There were 70 patients who belonged in the incident cohort. Of the seventy patients in 25 were men and 45 were women. Mean age of the cohort was 33.33 (IQR: 25 – 42.25) years. Close to half the patients belonged to Tamil Nadu, one-third were from West Bengal and the remainder from the rest of the country. This distribution of patients from different parts of the country can be explained as we are a tertiary referral centre providing services to the whole country. About 10% patients were already on antitubercular therapy at the time of recruitment. 72% patients had exclusive peripheral lymph nodal involvement whereas 28% of these patients had additional organ system involvement. Other baseline characteristics are described in the table 8.

Table 8: Baseline characteristics of the incident cohort (N = 70)

Sex	Male – 25 Female – 45
Mean Age	33.33 (IQR: 25 – 42.25)
Patients previously treated with ATT	7 (10%)
Extralymph nodal involvement	19(27.1%)
Patient Residence • Tamil Nadu • West Bengal • Kerala • Karnataka • Others	 • 34 (48.6%) • 21 (30%) • 1 (1.4%) • 1 (1.4%) • 13 (18.6%)
Patients who were on ATT prior to presentation	7 (10%)

Clinical, Laboratory and Microbiological characteristics of these patients are summarized in table 9. Only 8 patients (11.4%) had a positive AFB smear from the lymph node biopsy. Xpert TB PCR was positive in 33 patients (47.14%) with none of them having Rifampicin resistance. There was eventual culture growth in 34 patients (48.57%). Amongst the patients in whom sensitivity results were available, 23 were pansensitive. However, there were 4 patients who had drug resistance to at least 1 drug, none of them

had multidrug resistant or extensively drug resistant Tuberculosis. Lymph node histopathology showed well-formed granulomas in 53 patients (75.71%) and necrosis in 60 patients (85.71%). Most commonly involved lymph nodes were cervical nodes. In most instances, there was only single node involvement. At presentation, we found 8 patients had tenderness and fluctuation, 1 had discharge and 5 had sinuses.

Table 9: Clinical, Laboratory, Microbiological and Histopathological characteristics of Patients in the Incident Cohort

Clinical characteristics	
Site of Lymphadenopathy • Right Cervical • Left Cervical • Right Axillary • Left Axillary • Inguinal	 • 48 • 35 • 4 • 1 • None
Average Lymph node size	3.594 X 2.807 cm
Signs of inflammation at presentation Tenderness Fluctuation Discharge Sinus	14/70 – 8 – 8 – 1 – 5

Laboratory Parameters	
Hemoglobin (median)	12.22 (IQR: 11.03 – 12.20)
Absolute Lymphocyte Count (median)	1587 (IQR: 1216 – 2231)
ESR (median)	36 (IQR: 16 -50)
CRP (median)	11.9 (IQR: 3.35 – 30.9)
CD4 (median)	662 (IQR: 476 – 822)
Microbiological Parameters	
AFB smear positive	8 (11.4%)
Culture Growth – Present	34(48.6%)
Xpert TB PCR – Positive	33 (47.1%)
Pansensitive organism	23
Resistance to one drug(Isoniazid, Streptomycin)	4
Histopathological characteristics	
Well-formed granulomas	53 (75.7%)
Presence of necrosis	60 (85.7%)

Histograms below show the distribution of Hemoglobin, Absolute Lymphocyte count, ESR and CD4 counts among the 70 patients in the prospective arm.

Figure 8: Distribution of Hemoglobin in the incident cohort

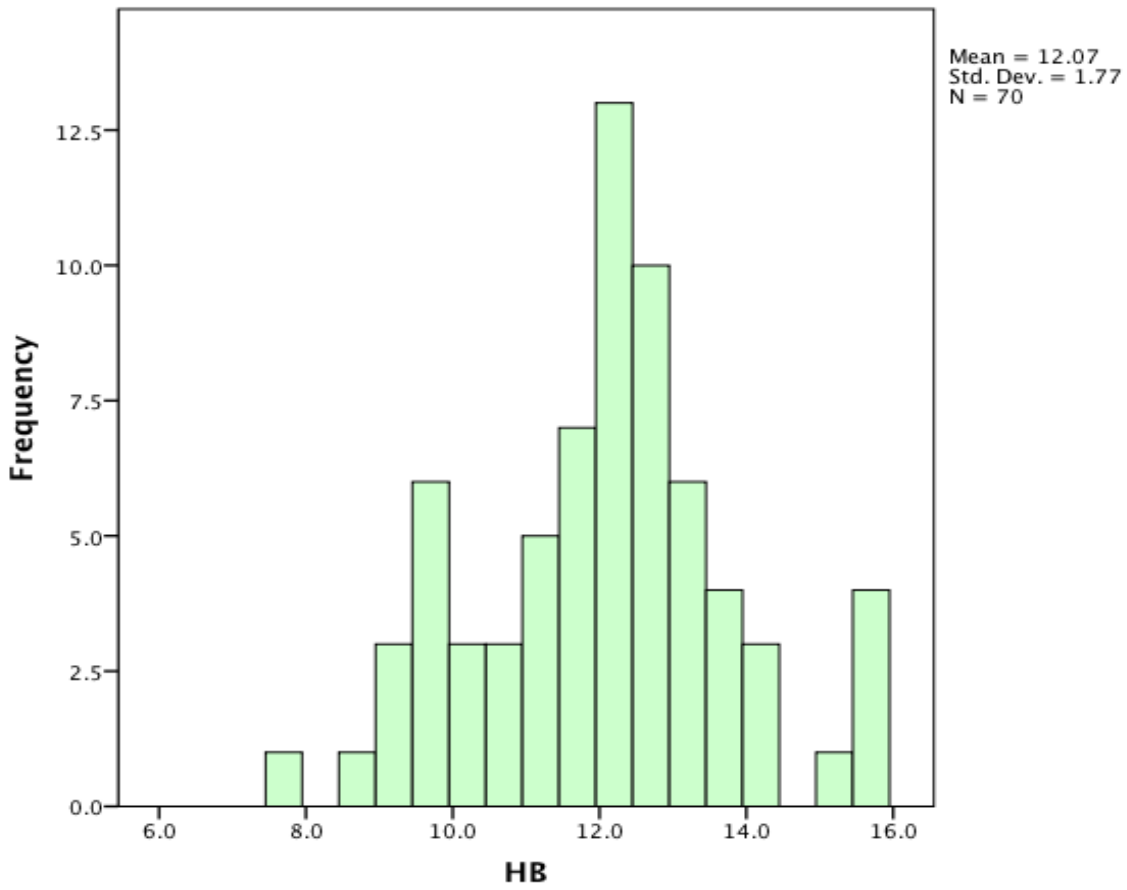


Figure 9: Distribution of Baseline Absolute Lymphocyte count in the incident cohort

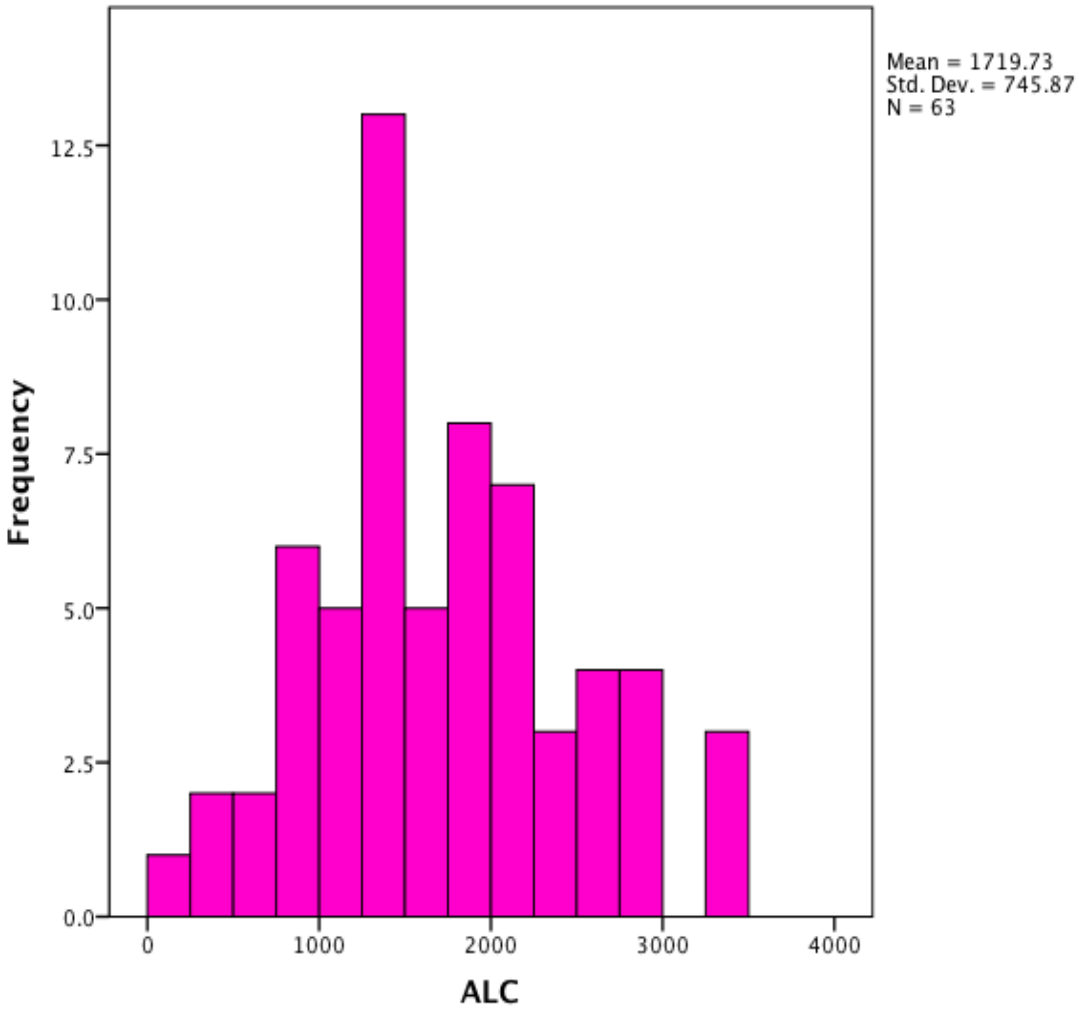


Figure 10: Distribution of Baseline ESR in the incident cohort

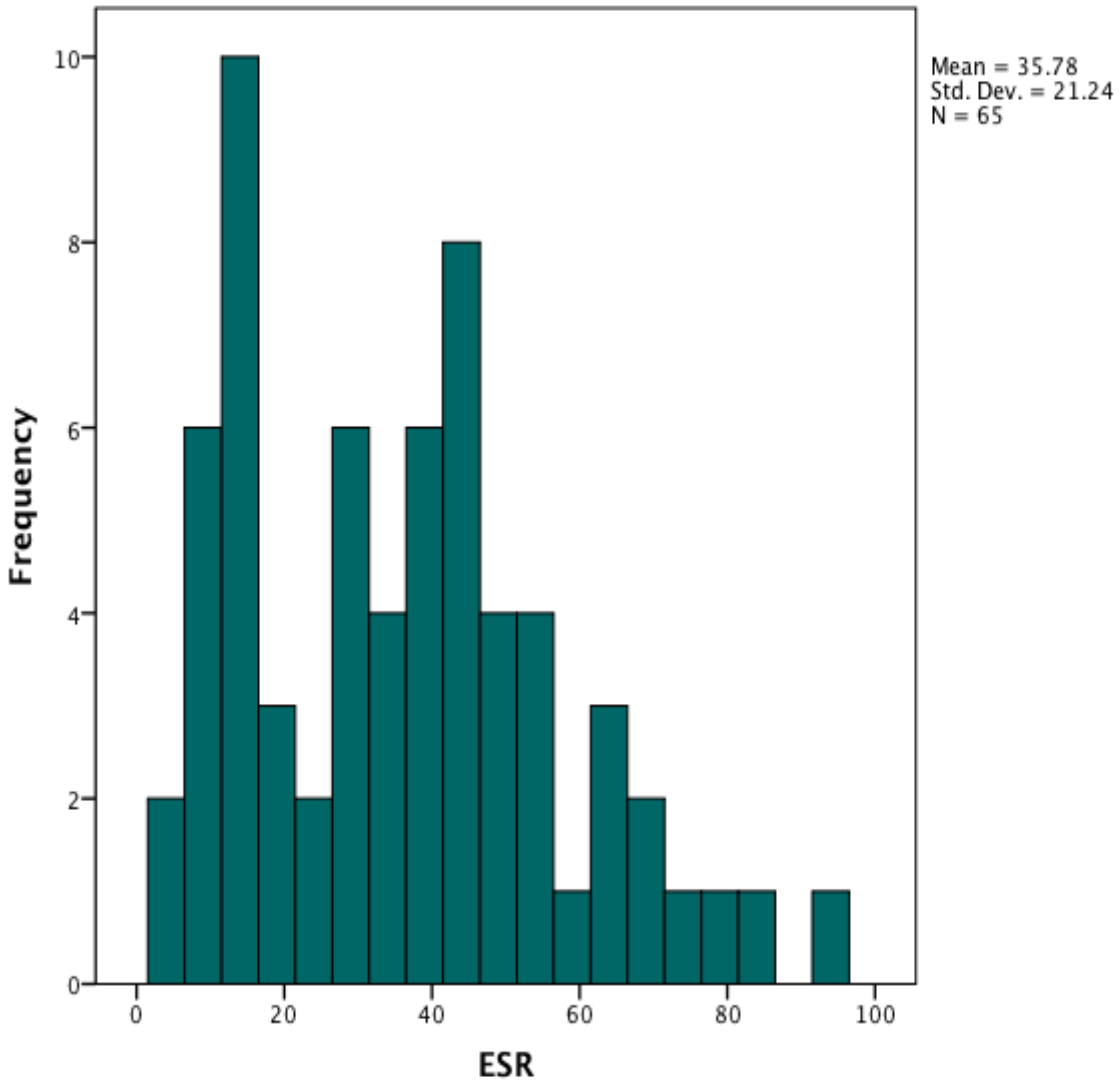


Figure 11: Distribution of baseline CRP in the incident cohort

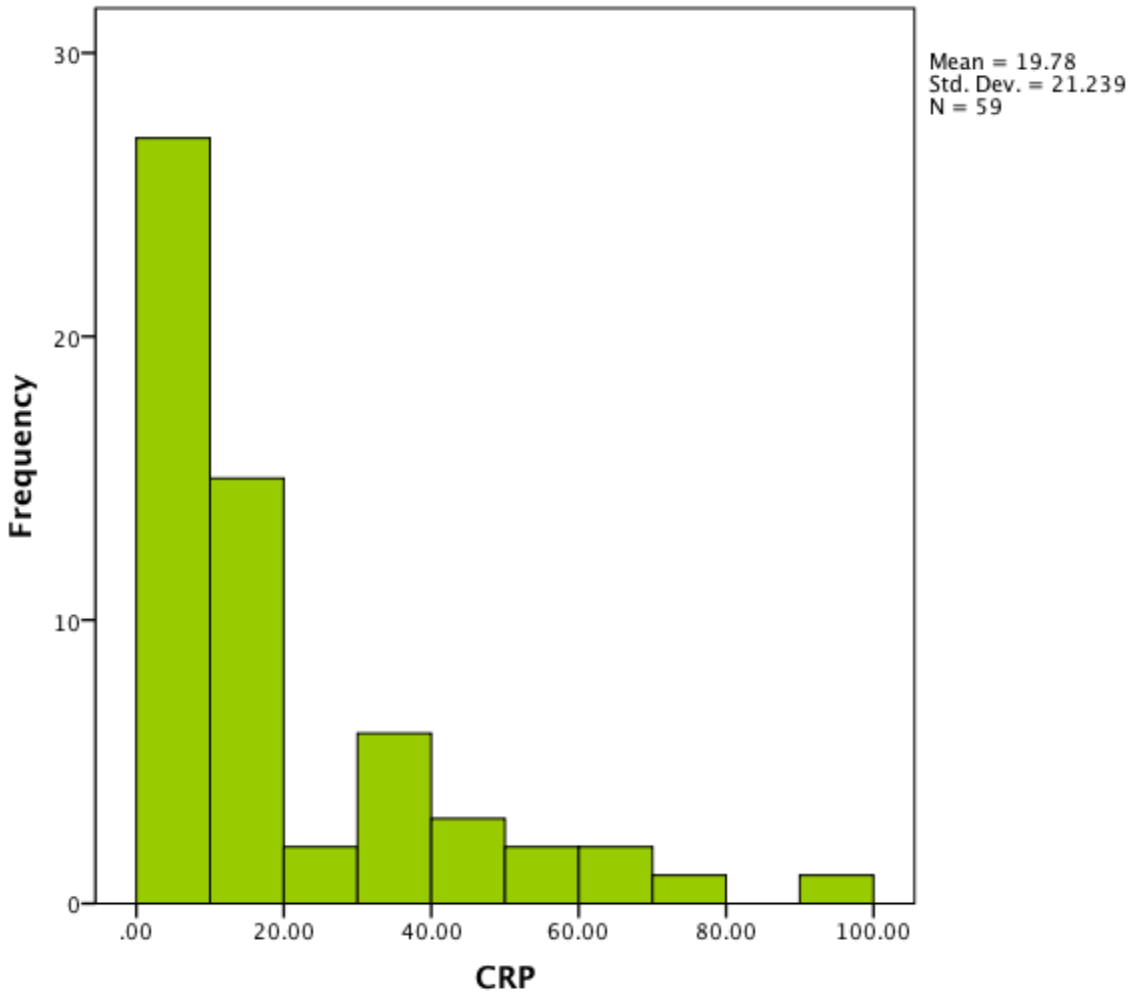
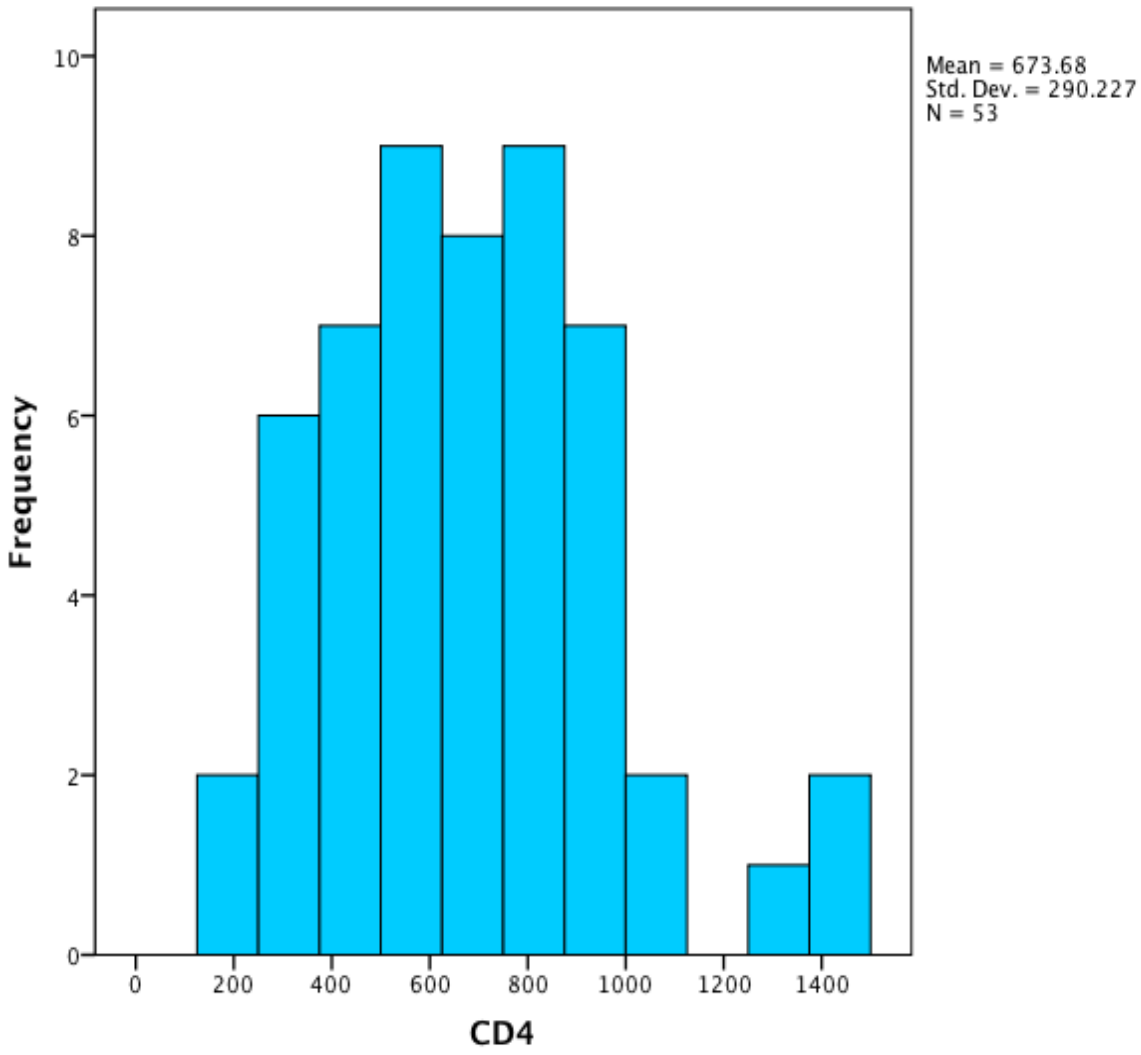


Figure 12: Distribution of baseline CD4 in the incident cohort



INCIDENT COHORT – PATIENTS WITH PR VERSUS THOSE WITHOUT PR

Four patients in the incident cohort developed paradoxical worsening. Clinical, laboratory, microbiological and histopathological features of these 4 patients were compared against the rest of the cohort.

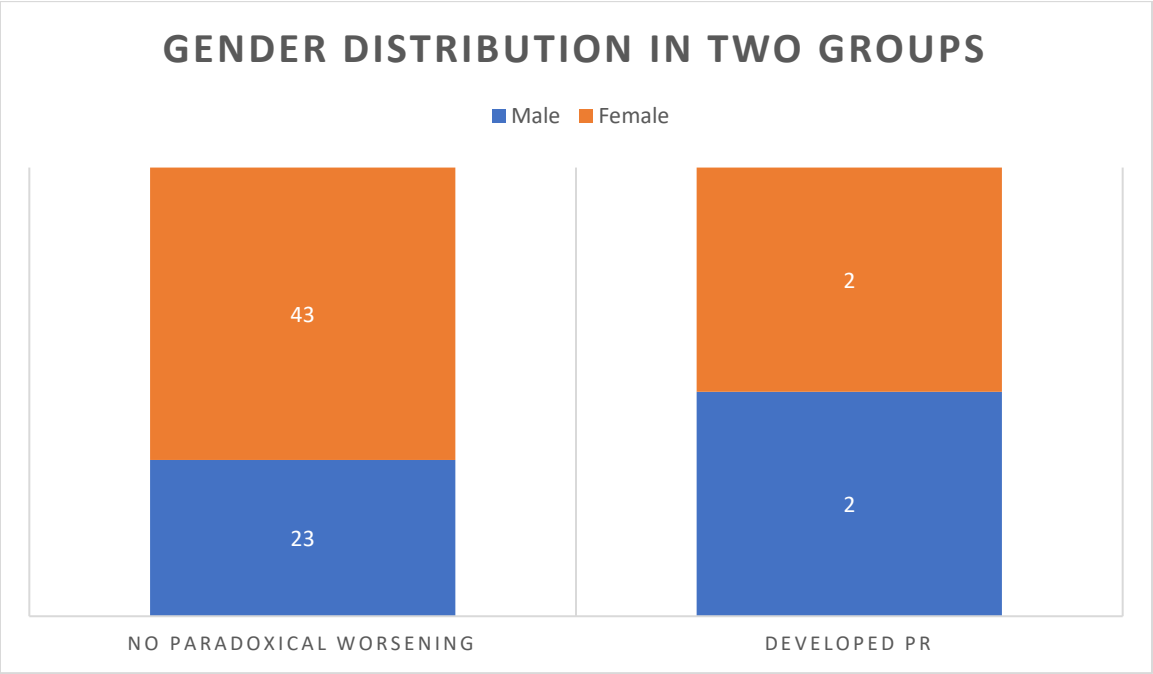


Figure 12: Gender distribution

At baseline, mean size of the lymph nodes were almost the same in the two groups. In terms of the signs of inflammation, we found that of the 4 patients, 1 (25%) had signs of inflammation at the baseline whereas of the 66 patients, 5 (7.6%) had signs of inflammation. In terms of laboratory parameters, median values of baseline hemoglobin, Absolute Lymphocyte count, ESR and CRP were similar in both the groups. Of the 4 patients who developed paradoxical worsening, we only had baseline CD4 count for one patient which was 719. Median CD4 count for the Non-PR arm was 657.

A comparison of the microbiological and histopathological characteristics of the two groups in the INCIDENT COHORT are summarized in table 10.

Table 10: INCIDENT COHORT – Patients who developed PR versus those who did not (BASELINE DATA)

Comparison of the baseline Clinical, Laboratory, Microbiological and Histopathological characteristics

	PR Arm N = 4	Non-PR arm N = 66
Baseline Clinical Characteristics		
Mean Age	31.75 (IQR: 23.5 – 38.75)	33.42 (IQR: 25 – 43)
Sex distribution (M/F)	2/2	23/43
Average size	2.8 X 1.97 cm	2.49 X 1.97 cm
Signs of inflammation (tenderness, fluctuation and sinus – any one of them)	1	5
Baseline Laboratory Characteristics		
Hemoglobin (median)	12.25 (IQR: 10.15 – 13.075)	12.2 (IQR: 11.025 – 13.0)
Absolute Lymphocyte Count (median)	1460	1628

	(Range: 664 – 1587) *	(IQR: 1229 – 2232)
ESR (median)	38.5 (IQR: 29.75 – 47.25)	32 (IQR: 15 – 50)
CRP(median)	16 (Range: 11.9 – 46.7) *	11.1 (IQR: 3.21 – 29.9)
CD4 (median)	719***	657 (IQR: 467 – 830)
Baseline Microbiological and Histopathological Characteristics		
AFB smear positive	1(25%)	7 (11.1%)
Xpert TB PCR	4(100%)	29 (49.2%)
Culture growth present	3(75%)	31 (50%)
Sensitivity 1. Pansensitive 2. Monoresistance	 3 0	 20 4
Baseline Biopsy 1. Well-formed Granulomas 2. Necrosis	 2(50%) 2(50%)	 51(77.3%) 58 (87.7%)

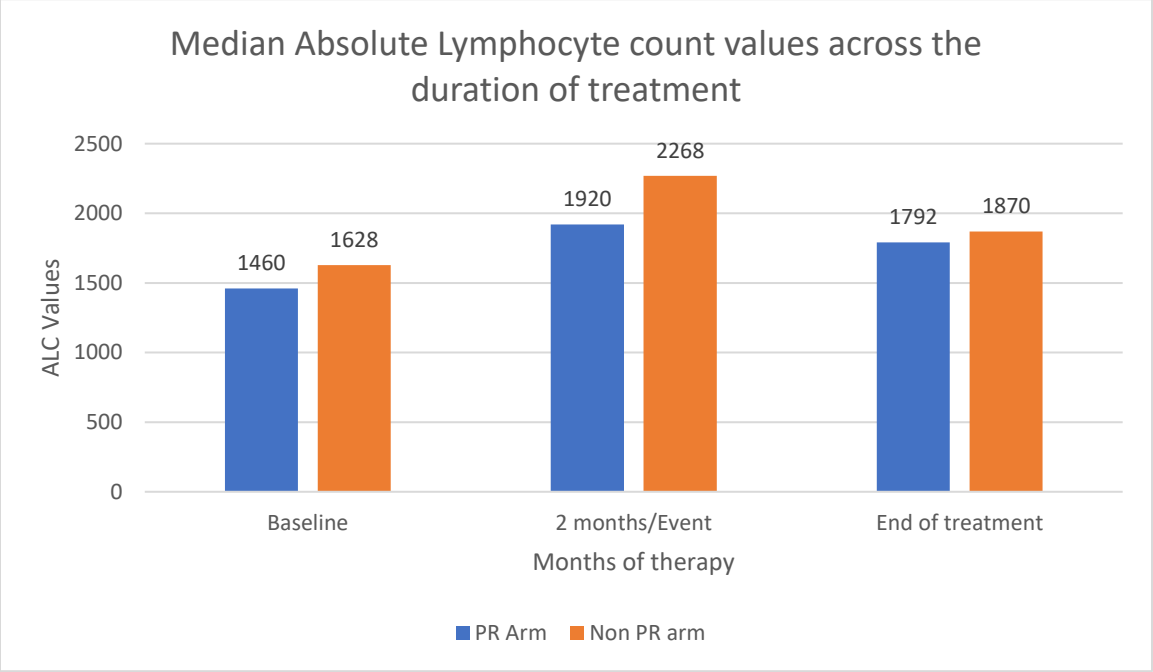
*1 missing

**2 missing

***3 missing

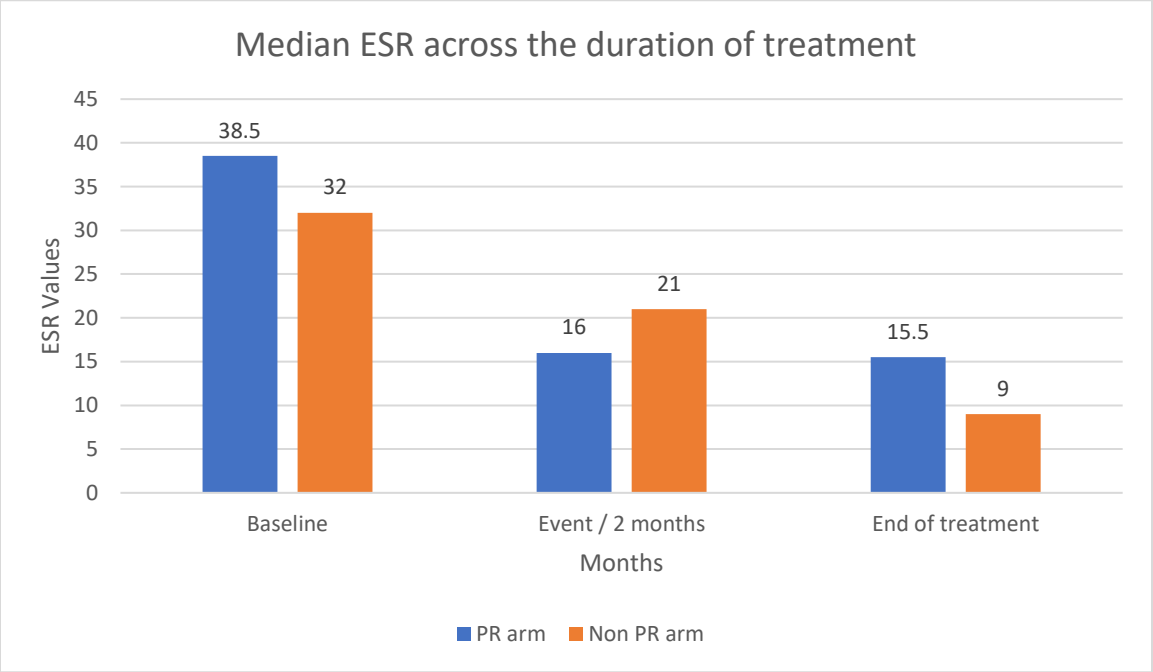
There was no significant change in the median absolute lymphocyte count at baseline, at the end of intensive phase, at the time of PR and at the end of continuation phase between the two groups.

Figure 13: Median ALC values across the duration of treatment



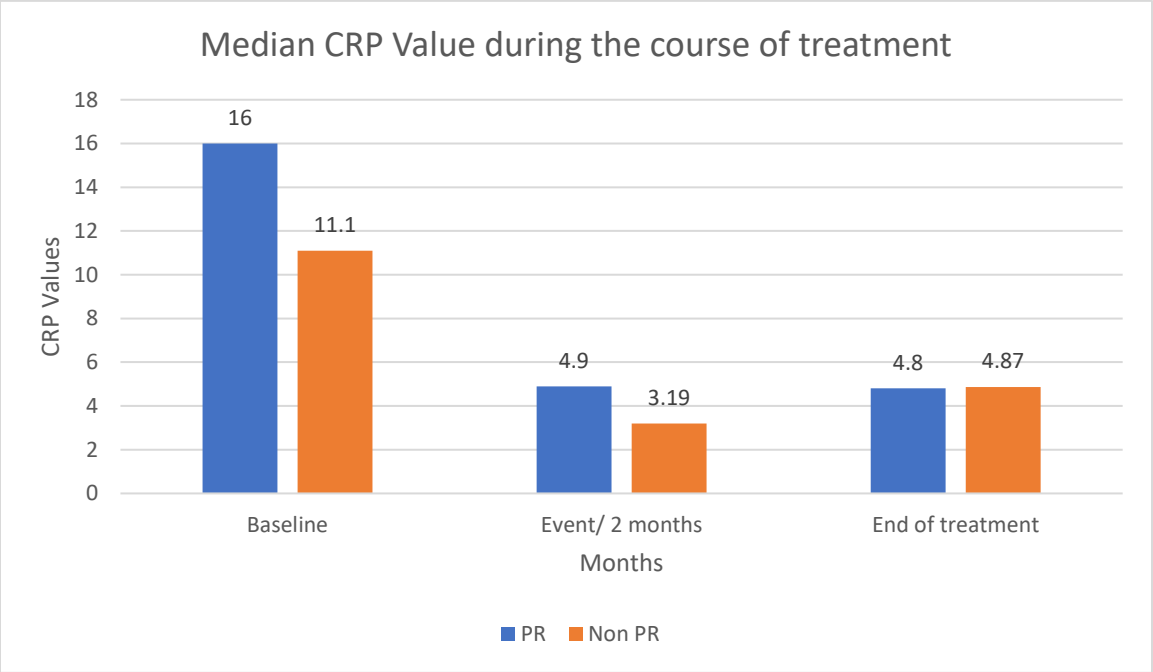
There was no significant change in the median values of ESR when measured at the end of intensive phase, at the time of PR and at the end of continuation phase between the two groups.

Figure 14: Median ESR across the duration of treatment



There was no significant change in the median values of CRP when measured at the end of intensive phase, at the time of PR and at the end of continuation phase between the two groups.

Figure 15: Median CRP across the duration of treatment



Amongst the patients in the PR group, re-biopsy was done in 2 of these patients, Xpert TB PCR was positive in both, AFB smear and culture was positive in 1 patient and the mycobacterium was pansensitive in that case. Histopathology of both the biopsies showed well-formed granulomas and necrosis.

Table no 11: Parameters at the time of paradoxical worsening in the incident cohort

Clinical, Laboratory, Microbiological and Histopathological characteristics at the time of Paradoxical Worsening in the Incident Cohort	
Average size	4.83(Range 3.5 – 7.0) cm
Hemoglobin (median)	12.1 (Range: 7.2 – 14.3) **
Absolute Lymphocyte Count (median)	1920 (Range: 206 - 1984) *
ESR (median)	16 (IQR: 6.25 – 48.25) &
CRP (median)	4.9 (Range: 3.02 – 7.23) *
CD4(median)	384 (Range: 78 – 691) **
Rebiopsy Status	Done – 2 Not Done – 2
AFB smear Status in the repeat biopsy sample	Positive – 1 Negative – 1
Xpert TB PCR Status in the repeat biopsy sample	Positive – 2 Negative -0
Culture growth status from the repeat biopsy sample	Present – 1 (Pansensitive) Absent – 1
Histopathology in repeat biopsy sample a. Well-formed granulomas b. Presence of necrosis	 a. 2 b. 2

*1 missing

**2 missing

***3 missing

FACTORS ASSOCIATED WITH PARADOXICAL WORSENING

Table 12: Univariate analysis of possible risk predictors at baseline

Variable	PR N = 4 (Median)	Non – PR N = 66 (Median)	Odds Ratio	95% CI	P value
Clinical Variables at baseline					
Age < 40	4	46	0.920	0.848 – 0.998	0.319
Sex (M/F)	2/2	23/43	1.87	0.27 – 14.154	0.613
Size >2 cm at baseline	1	7	0.57	0.05 – 6.27	0.530
Any signs of inflammation (discharge, tenderness, fluctuation)	1	5	4.067	0.35 – 46.65	0.30
Laboratory Variables at baseline					
Baseline Hemoglobin	12.25	12.2			0.874
Baseline ALC	1460	1628			0.294

Baseline ESR	38.5	32			0.683
Baseline CRP	16	11.1			0.325
Baseline CD4	719	657			0.906
Microbiological Variables at baseline					
AFB smear positivity at baseline	1	7	2.67	0.24 – 29.26	0.406
Xpert TB Positivity at Baseline	4	29	∞		0.115
Culture Positivity at baseline	3	31	3	0.29 – 30.44	0.614
Histopathological Variables at baseline					
Well-formed granulomas	2	51	0.216	0.027 – 1.701	0.171
Necrosis	2	58	0.069	0.008 – 0.626	0.039

Apart from presence of necrosis at baseline, no other baseline characteristics had any significant association with the occurrence of PR. Event numbers were not big enough for us to draw a reasonable conclusion.

PR COHORT

Baseline characteristics

During the process of recruitment, we identified 6 patients who satisfied the diagnostic criteria for PR (as mentioned in the case definition section) at presentation itself. Even though we did not have baseline data for these patients, we also followed up these patients to better understand the natural history of these patients especially because we had such a low incidence of paradoxical worsening in our incident cohort. Half of these patients had signs of inflammation and the rest presented with increase in the size of the node from before (based on patient history). Average size of the nodes at presentation was 3 X 3.3 cm. Baseline Hemoglobin, ALC, ESR, CRP and CD4 count were similar to the patients in the incident cohort.

Table 13: Baseline Characteristics of Patients presenting with Paradoxical Worsening

Hemoglobin (median)	11.35 (IQR: 10.68 – 13.25)
Absolute Lymphocyte Count (median)	1827(IQR: 1398.75 – 2336.25)
ESR (median)	31 (IQR: 18.75 – 59.25)
CRP (median)	20.8 (IQR: 3.16 – 36.95)
CD4 (median)	676 (IQR: 359 – 1230.50)
Average size	3 X 3.3 cm
AFB smear negativity	6(100%)
Culture Growth – Absent	6(100%)
Xpert TB PCR – Positive	3(50%)
Well-formed granulomas	5(83.3%)
Presence necrosis	4(66.7%)

The above table reiterates the fact that, paradoxical worsening is purely an inflammatory reaction, with the absence of Culture growth or AFB Smear positivity. Xpert TB PCR being a PCR, can detect even dead nonviable bacilli. Most of these patients had well-formed granulomas with necrosis.

Treatment Characteristics of the Entire Cohort (INCIDENT + PR COHORT)

We found that patients in this study received varying duration of antitubercular therapy (6 – 22 months) due to continued and worsening clinical symptoms. Mean duration of ATT consumption was 10 months with the median and mode being 9 months with an interquartile range from 8.25 to 10.75 months. Of the 76 patients, 34 patients completed antitubercular therapy while on follow-up in the study while the remaining were still completing their treatment course. 10 patients were lost to follow-up.

Mean time to Paradoxical worsening was 3.25 months (2 – 5 months). Amongst the 10 patients who had paradoxical worsening (6 patients in PR arm and 4 patients in the prospective arm), 8 patients had progression (worsening of the pre-existing lymph node) and 2 patients had dissemination (development of new nodes elsewhere).

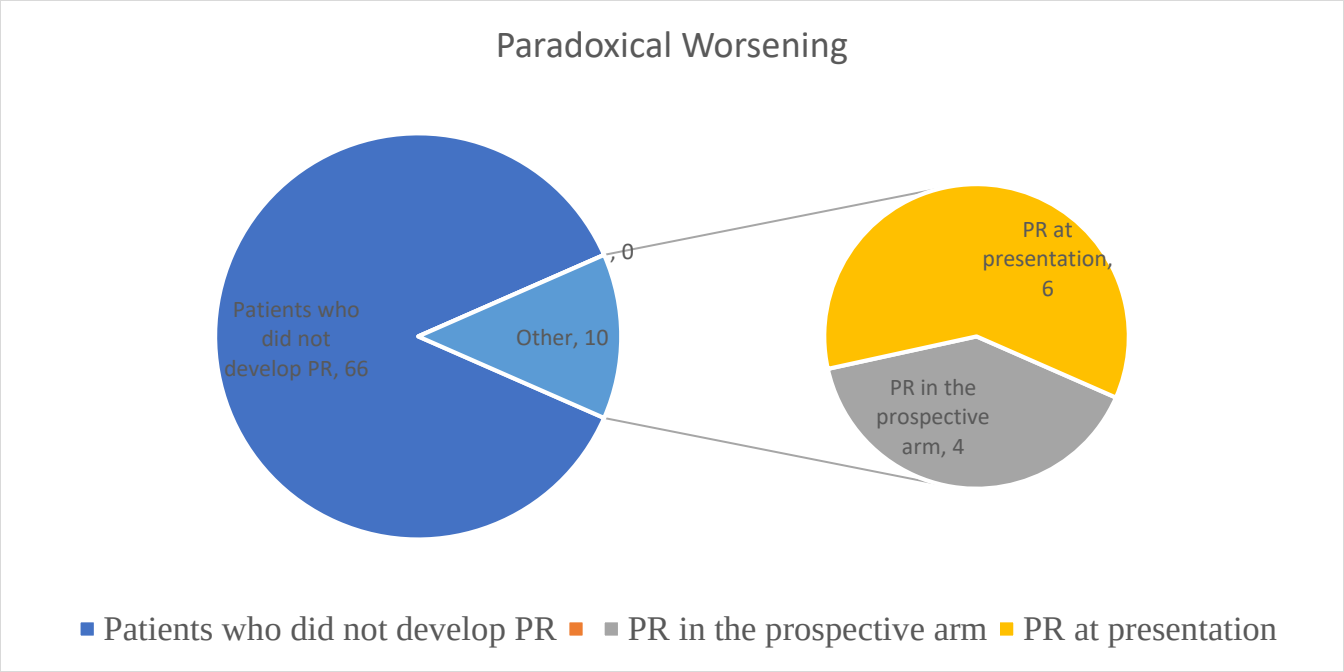


Figure 16: Patients with Paradoxical worsening in our cohort

Four patients underwent surgical debridement of which 3 underwent unilateral limited neck dissection and 1 underwent bilateral neck dissection with excision of sinuses. One patient received steroid, 3 patients received pentoxiphylline and 3 were managed conservatively with continuation of antitubercular therapy.

Table 14: TREATMENT CHARACTERISTICS OF PATIENTS WITH PARADOXICAL WORSENING (N = 10)

Total number of patients	76
Patients in the prospective Arm	70
PR in the incident cohort	6
PR Cohort	4
Mean size of node at the time of event	4.375 X 3 cm
Mean Time to Paradoxical Worsening	3.25 (2 – 5) months
Type of PR A. Progression B. Dissemination	 a) 8 b) 2
Management A. Conservative Management B. Surgical Management C. Steroid D. Pentoxiphylline	 a) 3 b) 4 c) 1 d) 3
Surgical Management	Unilateral Limited Neck Dissection (3) Bilateral Limited Neck Dissection with excision of Tuberculous Sinuses (1)

DISCUSSION

Paradoxical worsening is an exuberant inflammatory reaction, in a patient with tuberculosis on antitubercular therapy, characterized by initial improvement followed by development of clinical or radiological worsening in the absence of evidence of drug resistance or presence of another diagnosis(5). Paradoxical worsening has varied manifestation depending upon the site of involvement and the extent of involvement. It could be clinical worsening with development of an abscess or discharging sinus at the previously involved site, or it can involve completely new site or organ system for example development of pleural effusion, intrabdominal node or a mere lymph nodal enlargement elsewhere. Even though it's a well described entity in literature, understanding of this unique phenomenon is limited amongst the scientific community. Most of the studies on paradoxical worsening have come from western populations. It can be said that paradoxical worsening is underdiagnosed in our population, a population in which 2.2 million people develop Tuberculosis every year. Importance of diagnosing this phenomenon is mainly because most of these patients are diagnosed as drug resistant tuberculosis and are initiated on second line and third line antitubercular therapy. Thus, it becomes important to identify key features which will help a clinician recognize this entity and to identify baseline risk factors which could predict its occurrence.

This study involved a prospectively enrolled cohort of 76 patients with tuberculous lymphadenitis who presented to a tertiary care center in South India. Six of these patients presented with paradoxical worsening at their first visit itself. Four patients developed

paradoxical worsening in the incidence arm making an incidence of 5.71 % in our incident cohort. This incidence seems to be in keeping with the incidence described in literature for paradoxical worsening which ranges from 2.3 – 23% in literature (63).

Among the 10 patients who had paradoxical worsening, mean time to paradoxical worsening was 3.25 months ranging from 2 months to 5 months. In literature the mean time to occurrence of paradoxical worsening is varied but ranges from as short as 14 days to as long as 280 days(8,9,7,21,24,36).

Clinical manifestation of all our patients with paradoxical worsening was characterized by an increase in size of the lymph node, noticing new signs of inflammation (tenderness, fluctuation, sinus and discharge) in these nodes and development of nodes elsewhere. We found most of our patients had worsening at the primary site of tuberculosis rather than at a distant site. This description of paradoxical worsening is like what is described in literature.

We found that all the patients who developed paradoxical worsening had a negative AFB smear result and culture result at the time of the event except for 1 patient (described later). This emphasizes that in these patients this worsening is due to exaggerated host response rather than persistence of bacilli or drug resistance or treatment failure. However, Xpert TB PCR was positive in half of these patients possibly due to detection of non-viable bacilli. Thus, a positive Xpert TB PCR to be positive in this clinical setting should not be misconstrued as clinical failure or drug resistance leading to a change in therapy. Histopathologically, we identified that at the time of paradoxical worsening,

most of these patients had well-formed granulomas (87.5%) with necrosis (75%) reiterating the hosts immune response at these sites.

In literature, various risk predictors are described which we tried to look for in our analysis. We did not find any significant association with age, sex, lymph node size, signs of inflammation at baseline, associated extra-nodal disease, absolute lymphocyte count values at baseline, anemia at baseline, positive AFB smears and TB cultures at baseline. We found that presence of necrosis at baseline in the lymph node biopsy was the only variable associated with the occurrence of paradoxical worsening in the future. We must also consider, that the event occurred only in 4 patients amongst the 70 patients. Due to the small number of events, we may not have been able to identify any significant association which is a major limitation of our study.

An interesting finding in one of our patients, was that even after being diagnosed with paradoxical worsening on treatment, this patient developed 3 distinct new successive episodes of paradoxical worsening despite undergoing surgical management and being on appropriate antitubercular therapy. She developed repeated episodes of what events which could be termed as paradoxical worsening. However, repeated cultures grew pansensitive mycobacterium tuberculosis and she was never treated as Multidrug Resistant Tuberculosis and did receive a total of 18 months of antitubercular therapy. This was a rather interesting phenomenon as we felt there was a slow clearance of the tuberculosis in this case. This rather rare occurrence is described in literature by Geri et al, who identified 19 patients with paradoxical worsening, amongst whom 4 developed a

repeat event of paradoxical worsening. Most of the repeat events were at the same site as the first Paradoxical worsening and occurred within a mean duration of 2 months from the first episode and half of them required debridement/excision(7).

In our patients who developed paradoxical worsening, 4 out of 10 needed surgical debridement, which included Unilateral Limited Neck Dissection and Bilateral Limited Neck Dissection with excision of Tuberculous Sinuses. We also found that when we intervened surgically response was better and they did not seem to need adjunctive steroids or anti TNFα therapy. We had only one patient who received steroids. Pentoxiphylline was the only TNFα blocker which was used in our patients. We did not use monoclonal antibodies directed against TNFα or Thalidomide in any of our patients. Thus, surgical management seemed to eliminate the local reflex arc more effectively reducing the need for alternative therapy, reducing symptoms of the patients and the duration of anti-tubercular therapy.

CD4 count was used in our study as a surrogate marker for cell mediated immunity and for the ensuing inflammation. In patients with tuberculosis, CD4 counts having been found to be subnormal, as described in a study done in an Ethiopian population with HIV negative Pulmonary tuberculosis. This study showed that patients with lower CD4 count seemed to be more often sputum smear positive with significant wasting. With treatment, there was improvement in these parameters (64). Hence, we checked CD4 counts at baseline, however baseline CD4 count did not seem to predict the occurrence of paradoxical worsening. However at the time of paradoxical worsening, when we repeated

the CD4 count, we found that there may have been a decline in the CD4 count(61). Thus, paradoxical worsening may have an associated low CD4 count, although a definite association could not be established. The mechanism for this could be that exaggerated inflammatory reaction would results in increased sequestration of the cells to the sites of inflammation resulting in increased lowering in CD4 counts than that described in literature for HIV negative TB patients(62). In addition, there is increased homing of regulatory CD4 cells(CD4CD25+) cells in addition to T helper 1 and 2 cells to these sites especially in the setting of exaggerated inflammation(38). Literature suggests a drop in the CD4 counts of 100 cells/μL in patients with HIV negative Tuberculosis as compared to control(63). We suggest her that, CD4 counts do drop in a patient with tuberculosis, but it drops even further in patients who eventually develop paradoxical worsening.

Thus, putting all the above findings together, we can infer that a patient presenting with worsening symptoms suggestive of paradoxical worsening can be differentiated from drug resistance, poor drug absorption or treatment failure with microbiological and histopathological clues of a negative AFB smear, negative tissue culture, low CD4 count(<500) and well-formed granulomas with necrosis. This would need further validation for use in future research and clinical practice.

LIMITATIONS

There were a few limitations in this study. Firstly, we did not achieve the sample size which we intended to, due to slow recruitment. However, to the best of our knowledge this would be one of the first studies which looked into the paradoxical worsening in a prospective manner, as most of the previous studies have been retrospective. Therefore, this study, despite its small sample size, provides a scaffolding for further research for this phenomenon, especially in identifying risk factors, predictors and treatment options.

Secondly, the number of events which occurred in this cohort of patients were only 4, hence we were unable to come up with predictors of tuberculous lymphadenitis

Thirdly, as we had to follow-up patients at 2 months and end of treatment (6 or 9 months), we found it especially difficult to do the same as patients were from far off places, and usually most of our non-resident patients continue further care at their hometown. Thus, we lost quite a few valuable follow-up results. We may have lost data on patients who developed subtle features of paradoxical worsening but did not seek medical attention as distance was an issue. Hence, we may be under estimating the incidence of paradoxical worsening in our study.

CONCLUSIONS

In conclusion, this prospective study done on patients with tuberculous lymphadenitis, identified that the incidence of PR in our cohort was 5.71%. Patients with clinical features of paradoxical worsening had negative smear and culture suggesting an aberrant host response rather than drug resistance. This clinical entity needs to be publicized to prevent unnecessary, toxic and expensive empirical 2nd line Anti tubercular treatment. Our patients seem to respond better to surgical debridement rather than medical therapy possibly due to elimination of the local inflammatory reflex arc.

Future research must be aimed at ensuring larger number of patient recruitment with emphasis on good follow-up by using the same model as we used in this study in terms of the measurement of lymph node, laboratory assessment at each visit. A robust study with larger numbers in India would help identify predictors for this phenomenon applicable to the Indian setting.

In addition, studies must be done to elucidate the pathogenesis of this phenomenon, even though there are proposed theories, but none of them have been conclusively proven. We must perform studies to look at the cytokine response at baseline, end of intensive phase and end of treatment. We must understand that our understanding of tuberculosis pathogenesis is still at its nascent stage and more research is needed for the same.

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ANNEXURES

Annexure – 1: IRB Approval

OFFICE OF RESEARCH
INSTITUTIONAL REVIEW BOARD (IRB)
CHRISTIAN MEDICAL COLLEGE, VELLORE, INDIA

Dr. B.J. Prashantham, M.A., M.A., Dr. Mis (Clinical)
Director, Christian Counseling Center,
Chairperson, Ethics Committee.

Dr. Alfred Job Daniel, D Ortho MS Ortho DNB Ortho.
Chairperson, Research Committee & Principal

Dr. Biju George, MBBS., MD., DM
Deputy Chairperson,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

May 24, 2016

Dr. Akhil R,
PG Registrar
Department of Medicine
Christian Medical College,
Vellore - 632 004.

Sub: **Fluid Research Grant NEW PROPOSAL:**

To determine the clinical and laboratory risk predictors of paradoxical reaction in a cohort of patients with tuberculous lymphadenitis during the course of treatment.

Dr. Akhil R (Employment Number: 29446), Post Graduate Registrar, Medicine, Dr Priscilla Rupali (Employment Number: 14371), Infectious Diseases, Dr Alice Joan Mathuram (28529), Dr Vijay Prakash Turaka, 28591, Dr Soumya Sathyendra, 28181, Dr OC Abraham, 05638, Dr Ramya I, 31571, Medicine , Dr Visalakshi Jeyaseelan, 31093, Biostatistics , Dr Pranay Gaikwad, 31224, Surgery.

Ref: IRB Min No: 10021 [OBSERV] dated 04.04.2016,

Dear Dr. Akhil R,

I enclose the following documents:-

1. Institutional Review Board approval 2. Agreement

Could you please sign the agreement and send it to Dr. Biju George, Addl. Vice Principal (Research), so that the grant money can be released.

With best wishes,

Dr. Biju George
Secretary (Ethics Committee)
Institutional Review Board

Dr. BIJU GEORGE
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SECRETARY - (ETHICS COMMITTEE)
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Dr. B.J. Prashantham, M.A., M.A., Dr. Min (Clinical)
Director, Christian Counseling Center,
Chairperson, Ethics Committee.

Dr. Alfred Job Daniel, D Ortho MS Ortho DNB Ortho.
Chairperson, Research Committee & Principal

Dr. Biju George, MBBS., MD., DM
Deputy Chairperson,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

May 24, 2016

Dr. Akhil R,
PG Registrar
Department of Medicine
Christian Medical College,
Vellore 632 004.

Sub: **Fluid Research Grant NEW PROPOSAL:**
To determine the clinical and laboratory risk predictors of paradoxical reaction in a cohort of patients with tuberculous lymphadenitis during the course of treatment.

Dr. Akhil R (Employment Number: 29446), Post Graduate Registrar, Medicine, Dr Priscilla Rupali (Employment Number: 14371), Infectious Diseases, Dr Alice Joan Mathuram (28529), Dr Vijay Prakash Turaka, 28591, Dr Soumya Sathyendra, 28181, Dr OC Abraham, 05638, Dr Ramya I, 31571, Medicine , Dr. Visalakshi Jeyaseelan, 31093, Biostatistics , Dr Pranay Gaikwad, 31224, Surgery.

Ref: IRB Min No: 10021 [OBSERV] dated 04.04.2016

Dear Dr. Akhil R,

The Institutional Review Board (Blue, Research and Ethics Committee) of the Christian Medical College, Vellore, reviewed and discussed your project titled "To determine the clinical and laboratory risk predictors of paradoxical reaction in a cohort of patients with tuberculous lymphadenitis during the course of treatment" on April 04 2016.

The Committee reviewed the following documents:

1. IRB Application format
2. Patient Information Sheet and Informed Consent Form (English, Tamil, Hindi, Bengali, Telugu)
3. Cvs of Drs. Akhil, Alice, Abraham, Priscilla Rupali, Ramya, Ravikar, Soumya,
4. No. of documents 1 - 3.

The following Institutional Review Board (Blue, Research & Ethics Committee) members were present at the meeting held on April 04th 2016 in the CREST/SACN Conference Room, Christian Medical College, Bagayam, Vellore - 632002.

Ethics Committee Blue, Office of Research, 1st Floor, Carman Block, Christian Medical College, Vellore, Tamil Nadu 632 002
Tel: 0416 – 2284294, 2284202 Fax: 0416 – 2262788, 2284481 E-mail: research@cmcvellore.ac.in

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Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

Name	Qualification	Designation	Affiliation
Dr. Biju George	MBBS, MD, DM	Professor, Haematology, Research), Additional Vice Principal , Deputy Chairperson (Research Committee), Member Secretary (Ethics Committee), IRB, CMC,Vellore	Internal, Clinician
Dr. Anuradha Rose	MBBS, MD, MHSC (Bioethics)	Associate Professor, Community Health, CMC, Vellore	Internal, Clinician
Dr. Jayaprakash Muliyil	BSc, MBBS, MD, MPH, Dr PH (Epid), DMHC	Retired Professor, Vellore	External, Scientist &Epidemiologist
Rev. Joseph Devaraj	BSc, BD	Chaplaincy Department, CMC, Vellore	Internal, Social Scientist
Ms. Grace Rebekha	M.Sc., (Biostatistics)	Lecturer, Biostatistics, CMC, Vellore	Internal, Statistician
Dr. Visalakshi. J	MPH, PhD	Lecturer, Biostatistics, CMC, Vellore	Internal, Statistician
Dr. Mathew Joseph	MBBS, MCH	Professor, Neurosurgery, CMC, Vellore	Internal, Clinician
Mr. Samuel Abraham	MA, PGDBA, PGDPM, M. Phil, BL.	Sr. Legal Officer, CMC, Vellore	Internal, Legal Expert
Mrs. Pattabiraman	BSc, DSSA	Social Worker, Vellore	External, Lay Person
Dr. B. J. Prashantham	MA(Counseling Psych MA(Theology), Dr. Min(Clinical Counselling)	Chairperson, Ethics Committee, IRB. Director, Chri Counseling Centre, Vellore	External, Social Scientist
Dr. Rajesh Kannangai	MD, PhD.	Professor, Clinical Virology, CMC, Vellore	Internal, Clinician
Dr. Thomas V Paul	MD, DNB(Endo), Phd(Endo)	Professor, Endocrinology, CMC, Vellore	Internal, Clinician
Mrs. Emily Daniel	MSc Nursing	Professor, Medical Surgical Nursing, CMC, Vellore	Internal, Nurse
Dr. Sathish	MBBS, MD, DCH	Professor, Child Health, CMC, Vellore	Internal, Clinician

Ethics Committee Blue, Office of Research, 1st Floor, Carman Block, Christian Medical College, Vellore, Tamil Nadu 632 002
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Deputy Chairperson,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

Mr. C. Sampath	BSc, BL	Advocate, Vellore	External, Legal Expert
Dr. Balamugesh	MBBS, MD(Int Med), DM, FCCP (USA)	Professor, Pulmonary Medicine, CMC, Vellore	Internal, Clinician
Dr. Inian Samarasam	MS, FRCS, FRACS	Professor, Surgery, CMC, Vellore	Internal, Clinician
Dr. Vivek Mathew	MD (Gen. Med.) DM (Neuro) Dip. NB (Neuro)	Professor, Neurology, CMC, Vellore	Internal, Clinician
Dr. Ratna Prabha	MBBS, MD (Pharma)	Associate Professor, Clinical Pharmacology, CMC, Vellore	Internal, Pharmacologist

We approve the project to be conducted as presented.

Kindly provide the total number of patients enrolled in your study and the total number of with drawals for the study entitled: "To determine the clinical and laboratory risk predictors of paradoxical reaction in a cohort of patients with tuberculosis lymphadenitis during the course of treatment" on a monthly basis. Please send copies of this to the Research Office (research@cmcvellore.ac.in).

Fluid Grant Allocation:

A sum of 1,00,000/- INR (Rupees One Lakh Only) will be granted for 2 years. 50,000/- INR (Rupees Fifty Thousand only) will be granted for 12 months as an Ist Installment. The rest of the 50,000/- INR (Rupees Fifty Thousand only) each will be released at the end of the first year as 2 nd Installments.

Yours sincerely

Dr. Biju George
Secretary (Ethics Committee)
Institutional Review Board

Dr. BIJU GEORGE
MBBS., MD., DM.
SECRETARY - (ETHICS COMMITTEE)
Institutional Review Board,
Christian Medical College, Vellore - 632 002.

IRB Min No: 10021 [OBSERV] dated 04.04.2016 4 of 4

Annexure 2 – PATIENT INFORMATION SHEET

Title of Research Project: *To determine the clinical and laboratory risk predictors of paradoxical reaction in a cohort of patients with Tuberculous lymphadenitis during the course of treatment.*

PATIENT INFORMATION SHEET

STUDY TITLE: To determine the clinical and laboratory risk predictors of paradoxical reaction in a cohort of patients with Tuberculous lymphadenitis during the course of treatment.

STUDY LOCATION: Department Of Medicine, Christian Medical College

1. **What is the background and purpose of study?**
   Tuberculosis remains one of the deadliest communicable diseases of the world. It has been found that certain patients after being started on anti-tubercular therapy, develop a worsening of their existing disease. This is what we are terming as paradoxical reaction. This is a common entity described in HIV positive patients. Occurrence of this phenomenon in HIV negative patients is well described in literature, but however not studied. In this study we intend to look at proportion of our patients who develop this phenomenon while on treatment. This can be crucial especially because knowledge of this entity would prevent the injudicious use of toxic and more expensive second line anti-tubercular therapy.

2. **What will I have to do if I have to take part in the study?**
   You will be expected to sign a simple consent form. You will be expected to provide your phone number to keep you in contact during the duration of therapy. You will also be explained regarding specific signs suggestive of paradoxical reaction so that you may inform us regarding the same. You will also have phone number of a Physician who is conducting the study. Before initiating anti tubercular therapy, some baseline blood investigations will be done. Subsequently, at the end of 2 months, 6 months and at the

time of occurrence of paradoxical reaction you will be asked to undergo blood investigations. All blood investigations pertaining to the study will be done free of cost for you. Blood samples drawn from you will be stored and will be used for determining host polymorphisms at

3. **Will there be any study related injuries?**
   No study related injuries are expected from this study as we are not planning any intervention in this study. You will be receiving standard treatment as that of other patients who are not part of the study.

4. **Can I withdraw from the study if I wish to?**
   You are free to withdraw from the study at any point in time. Your decision is entirely voluntary and your consent or withdrawal will not affect your treatment in the hospital.

5. **Will the information be kept confidential?**
   The patient's identity will not be disclosed. All data will be acquired from the Clinical Workstation of the institution. Only the doctors evaluating the reports will know the identity of the patient. If you give us permission by signing on the form below, the details of the patient along with those of other patients who take part in this study will may be published in a medical journal. Thank you for reading this information. Now please read through the attached consent form. If you have any questions, please contact the team below:
   Dr Akhil R, PG Resident, Department of Medicine, Christian Medical College Vellore

Title of Research Project: *To determine the clinical and laboratory risk predictors of paradoxical reaction in a cohort of patients with Tuberculous lymphadenitis during the course of treatment.*

Phone: 9894255318
Email: akhilrk1989@gmail.com

Annexure 3 – Patient consent Sheet

Format for Informed Consent Form for Subjects

Informed Consent form to participate in a research study

Study Title: To determine the clinical and laboratory risk predictors of paradoxical reaction in a cohort of patients with Tuberculous lymphadenitis during the course of treatment.

Study Number: _____

Subject's Initials: _____ **Subject's Name:** _____

Date of Birth / Age: _____

(Subject)

(i) I confirm that I have read and understood the information sheet dated _____ for the above study and have had the opportunity to ask questions. [ ]

(ii) I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. [ ]

(iii) I understand that ***the Sponsor of the clinical trial, others working on the Sponsor's behalf (delete as appropriate)***, the Ethics Committee and the regulatory authorities will not need my permission to look at my health records both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the trial. I agree to this access.

However, I understand that my identity will not be revealed in any information released to third parties or published. [ ]

(iv) I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s). [ ]

(v) I agree to take part in the above study. [ ]

Signature (or Thumb impression) of the Subject/Legally Acceptable

Date: _____/_____/_____

Signatory's Name: _____ Signature:

Or

Representative: _____

Date: _____/_____/_____

Signatory's Name: _____

Signature of the Investigator: _____

Date: _____/_____/_____

Study Investigator's Name: _____

Signature or thumb impression of the Witness: _____

Date: _____/_____/_____

Name & Address of the Witness: _____

Annexure 4 – Data Abstraction Sheet

Title of Research Project: *To determine the clinical and laboratory risk predictors of paradoxical reaction in a cohort of patients with Tuberculous lymphadenitis during the course of treatment*

DATA EXTRACTION FORM FOR TBPR STUDY

Name: Age: Occupation:

Hospital Number:

Date of enrollment:

Day Month Year

Sex:

1. Male	2.FEMALE

Place:

1. TN	2. AP	3. WB	4. Kerala	5. Karnataka	6. others

GENERAL DATA REGARDING THE PATIENT AT INITIAL DIAGNOSIS

WAS THE PATIENT PREVIOUSLY TREATED WITH ANTITUBERCULAR THERAPY?

1. Yes	2. No

If Yes, Drugs with its dosage:

Drug	Dose taken by patient	Ideal dose per kg	Actual dose needed
Isoniazid			
Rifampicin			
Pyrazinamide			
Ethambutol			
Streptomycin			
Others			

Mention the dose of other drug consumed by the patient if any and whether or not it was adequate

HIV Status: Yes/No

1. Yes	2. No

History of use of steroids for treatment?

1. Yes	2. No

Title of Research Project: *To determine the clinical and laboratory risk predictors of paradoxical reaction in a cohort of patients with Tuberculous lymphadenitis during the course of treatment*

Other sites involved apart from lymph node?

1. Lung	2. Liver	3. GI	4. CNS	5. Genitourinary	6. Others

Is this a case of Paradoxical reaction at first presentation?

7. Yes	8. No	9. Not sure

Please Note

If Yes, please continue onto **section II of the extraction form**

If No, please continue onto **section I**

If Not sure, fill up both forms, decide whether its PR or not later on in consultation with the guide and lab investigations

Title of Research Project: *To determine the clinical and laboratory risk predictors of paradoxical reaction in a cohort of patients with Tuberculous lymphadenitis during the course of treatment*

SECTION I

LYMPH NODES INVOLVED:

Site	Right		Left	
Cervical	1. Yes	2. No	1. Yes	2. No
Axillary	1. Yes	2. No	1. Yes	2. No
Inguinal	1. Yes	2. No	1. Yes	2. No

AFB SMEAR: Positive/ Negative

1. Positive	2. Negative

CULTURE: Growth present/Absent

1. Present	2. Absent

XPERT TB PCR Results

1. Positive	2. Negative

Sensitivity: Pan sensitive / Resistant

1. Pan sensitive	2. Resistant

GRANULOMAS IN BIOPSY: Present/Absent

1. Well formed	2. Illformed

Presence of necrosis

1. Present	2. Absent

At diagnosis

Site	No.	Size	Tenderness		Fluctuation		Discharge		Sinus	
RIGHT C			1. Yes	2. No	1. Yes	2. No	1. Yes	2. No	1. Yes	2. N
LEFT C			1. Yes	2. No	1. Yes	2. No	1. Yes	2. No	1. Yes	2. N

Title of Research Project: *To determine the clinical and laboratory risk predictors of paradoxical reaction in a cohort of patients with Tuberculous lymphadenitis during the course of treatment*

RIGHT A			1. Yes	2. No	1. Yes	2. No	1. Yes	2. No	1. Yes	2. N
LEFT A			1. Yes	2. No	1. Yes	2. No	1. Yes	2. No	1. Yes	2. N
RIGHT I			1. Yes	2. No	1. Yes	2. No	1. Yes	2. No	1. Yes	2. N
LEFT I			1. Yes	2. No	1. Yes	2. No	1. Yes	2. No	1. Yes	2. N

Lab tests at diagnosis

Hb:

ALC:

ESR:

CRP:

CD4:

AT THE TIME OF THE EVENT

SECTION (A): ASSESSMENT OF THE EVENT

Event: Yes/ No

Progression: Yes/No

Size at the event:

Dissemination: Yes/No

New site:

Fluctuation: Yes/No

Discharge: Yes/No

Sinus formation: Yes/No

Hb:

ALC:

ESR:

CRP:

SECTION (B): RULING OUT OTHER DIFFERENTIALS

Drug compliance assessment

Number of days of skipped pills:

Title of Research Project: *To determine the clinical and laboratory risk predictors of paradoxical reaction in a cohort of patients with Tuberculous lymphadenitis during the course of treatment*

Blister count:

Last day of consumption of medications:

Drugs with its dosage:

Drug	Dose taken by patient	Ideal dose per kg	Actual dose needed
Isoniazid			
Rifampicin			
Pyrazinamide			
Ethambutol			
Streptomycin			
Others			

Mention the dose of other drug consumed by the patient if any and whether or not it was adequate

Has the dose of drug been adequate?

1. Yes	2. No

Ruling out drug resistance

Follow-up previous culture and sensitivity

1. Pan sensitive	2. Resistant

Rebiopsy done

Yes	No

If No, please site the reason,

a. Too small a node
b. Surgically not feasible
c. Patient did not consent

Rebiopsy resultS:

AFB SMEAR: Positive/ Negative

1. Positive	2. Negative

Title of Research Project: *To determine the clinical and laboratory risk predictors of paradoxical reaction in a cohort of patients with Tuberculous lymphadenitis during the course of treatment*

CULTURE: Growth present/Absent

1. Present	2. Absent

Sensitivity: Pan sensitive / Resistant

1. Pan sensitive	2. Resistant

XPERT TB PCR Results

1. Positive	2. Negative

GRANULOMAS IN BIOPSY: Present/Absent

1. Positive	2. Negative

Presence of necrosis

1. Present	2. Absent

Poor drug absorption assessement

History of vomiting : Yes/No

History of diarrhea : Yes/No

History of alcohol usage while on ATT: Yes/No

AT THE END OF INTENSIVE PHASE

Progression: Yes/No

Dissemination: Yes/No

Fluctuation: Yes/No

Discharge: Yes/No

Sinus formation: Yes/No

Size:

New site:

Title of Research Project: *To determine the clinical and laboratory risk predictors of paradoxical reaction in a cohort of patients with Tuberculous lymphadenitis during the course of treatment*

Hb:

ALC:

ESR:

CRP:

AT THE END OF CONTINUATION PHASE

Progression: Yes/No Size:

Dissemination: Yes/No New site:

Fluctuation: Yes/No

Discharge: Yes/No

Sinus formation: Yes/No

Hb:

ALC:

ESR:

CRP:

Title of Research Project: *To determine the clinical and laboratory risk predictors of paradoxical reaction in a cohort of patients with Tuberculous lymphadenitis during the course of treatment*

THIS PART OF THE DATA EXTRACTION FORM IS ONLY FOR THOSE PATIENTS WHO PRESENT WITH PARADOXICAL REACTION

LYMPH NODES INVOLVED:

Site	No.	Size	Tenderness		Fluctuation		Discharge		Sinus	
RIGHT C			3. Yes	4. No	3. Yes	4. No	3. Yes	4. No	3. Yes	4. N
LEFT C			3. Yes	4. No	3. Yes	4. No	3. Yes	4. No	3. Yes	4. N
RIGHT A			3. Yes	4. No	3. Yes	4. No	3. Yes	4. No	3. Yes	4. N
LEFT A			3. Yes	4. No	3. Yes	4. No	3. Yes	4. No	3. Yes	4. N
RIGHT I			3. Yes	4. No	3. Yes	4. No	3. Yes	4. No	3. Yes	4. N
LEFT I			3. Yes	4. No	3. Yes	4. No	3. Yes	4. No	3. Yes	4. N

AFB SMEAR: Positive/ Negative

1. Positive	2. Negative

CULTURE: Growth present/Absent

1. Present	2. Absent

Sensitivity: Pan sensitive / Resistant

1. Pan sensitive	2. Resistant

XPERT TB PCR Results

1. Positive	2. Negative

GRANULOMAS IN BIOPSY: Present/Absent

1. Positive	2. Negative

Presence of necrosis

1. Present	2. Absent

Title of Research Project: *To determine the clinical and laboratory risk predictors of paradoxical reaction in a cohort of patients with Tuberculous lymphadenitis during the course of treatment*

Drug compliance assessment

Number of days of skipped pills:

Blister count:

Last day of consumption of medications:

Drugs with its dosage: Please refer to first section(introduction)

Poor drug absorption assessement

History of vomiting : Yes/No

History of diarrhea : Yes/No

History of alcohol usage while on ATT: Yes/No

Lab tests at diagnosis

Hb:

ALC:

ESR:

CRP:

CD4:

Followup data in the aforementioned sections: end of intensive phase & end of continuation phase

Title of Research Project: *To determine the clinical and laboratory risk predictors of paradoxical reaction in a cohort of patients with Tuberculous lymphadenitis during the course of treatment*

Additional Questions

1. **Total duration of Antitubercular treatment taken**
    a. **From outside(In months):**
    b. **From CMC(In months):**

2. **What form of treatment for paradoxical reaction was given?**
    a. **Surgical treatment**
        i. **Surgical debridement**
        ii. **As part of biopsy**
    b. **Steroid**
        i. **Dosage**
        ii. **Duration**
    c. **Pentoxiphylline**
    d. **Conservative management(continuation of ATT)**

3. **What was the type of Paradoxical reaction?**
    a. **Progression**
    b. **Dissemination**

Annexure 5 – Data Sheet

axilllt	ingurt	ingult	afb	culture	xpert	rifr	sensit	biopsy	necrosis	rcno	rclsize	rcbsize	rctend	rcfluct	rcdis	rcsin	lcno	lclsize	lcbsize	lctend	lcfluct	lcdis	lcsin	rano	ralsize	rabsize	ratend
2	2	2															2	2.2	1.2	2	2	2	2				
2	2	2	2	2	2			1	1								1	3	2	2	2	2	2				
2	2	2	2	1	2		1	1	1	2	3	2.6	2	2	2	2											
2	2	2	2	2	1	2		1	1								99	3	4	2	2	2	2				
2	2	2	2	2	1	2		1	1								1	2	2	2	2	2	2	2	4	4	2
2	2	2	1	1	1	2	1	2	2	1	7	3.6	1	1	2	2											
2	2	2	2	1	1	2	1	1	2	1	3	2	2	2	2	2											
2	2	2	2	1	1	2	1	1	1	3	2.6	2	2	2	2	2	2	3.2	2.4	2	2	2	2				
2	2	2	2	1	2		1	1	2	1	2.4	2.1	2	2	2	2											
2	2	2	2	1				1	1	1	3	2.5	2	2	2	2											
2	2	2	2	2	2			1	1								1	2	2	2	2	2	2				
2	2	2	2	1	2		1	1	1	1	2	1	2	2	2	2											
2	2	2	2	1	1	2	1	1	1								99	2	1	2	2	2	2				
2	2	2								1	3	3	2	2	2	2	2	2	2	2	2	2	2				
2	2	2	2	1	2		1	2	1	1	2	2	2	2	2	2											
2	2	2	2	1	1	2	1	1	1	1	7.2	6.2	2	2	2	2											
2	2	2	2	1	2		1	1	1								99	4	3	2	2	2	2				
2	2	2	2	2	2			2	1	1	3	2.5	2	2	2	2											
2	2	2	2	1	1	2	1	1	1								99	7	5	1	1	2	2				
2	2	2	2	1	1	2	1	1	1								1	3	2.5	2	2	2	2				
2	2	2	2	1	1	2	1	1	1	99	1	1	2	2	2	2	99	2	2	2	2	2	2				
2	2	2	2	2	2			1	1								2	4.3	3.9	1	2	2	2				
2	2	2	2	2	1	2		1	1								2	2.1	1	2	2	2	2				
2	2	2	2	2	2			1	1	2	1	0.5	2	2	2	2											
2	2	2	1	2	1	2				1	4.7	1.2	1	2	2	1											
2	2	2	2	2	2			1	1	1	1	1	2	2	2	2	1	1	1	2	2	2	2				
2	2	2	2	2	1	2		1	1	99	3	2	2	2	2	1											
2	2	2	2	1	2		2	2	1	1	6.1	4.8	1	1	2	2											
1	2	2	2	2	1	2		2	1	1			2	2	2	2								1	1	1	2
2	2	2	2	2	2			1	1								1	3	2	2	1	2	2				
2	2	2	2	2	1	2		1	2								99			2	2	2	2				
2	2	2	2	1	2		1	1	1	1	3.4	2.5	2	2	2	2	99	1	1	2	2	2	2				
2	2	2	2	1	2		2	1	1								32	2.4	1.8	2	2	2	2				
2	2	2	2	1	1	2		2	2	1	3.3	3	1	1	2	2											
2	2	2	2	1	1	2	1	1	1	1	5	3	2	2	2	2											
2	2	2	2	1			1	1	1								1	2.4	2.2	2	2	2	2				
2	2	2	2	1			1	1	1	3	1	1	2	2	2	2											
2	2	2	2	2	2			1	1	1	2.6	2.3	2	2	2	2											
2	2	2	2	2	1	2		1	1	1	1.3	1	2	2	2	2	3	2.5	2.3	2	2	2	2				
2	2	2	2	2				1	1	1	3	2.4	2	2	2	2											
2	2	2	1		1	2				1	4.5	1.5	2	2	2	2	1	2.6	2.5	2	2	2	2				
2	2	2	2	2	2			1	1	3	2	2	2	2	2	2	2	2	2	2	2	2	2				
2	2	2	2	2	2			1	1																		
2	2	2	2	1	1	2	1	1	1	99	1	1	2	2	2	1											
2	2	2	2	2	2			1	1	3	2	1.6	2	2	1	1	3	2	2	2	2	2	2				
2	2	2	2	2	1	2		1	1	1			2	1	2	2											
2	2	2	2	1	1	2		1	1	1	7	4	2	2	2	2											
2	2	2	2	2	2			1	1	2	3	2	2	2	2	2											
2	2	2	2	2	2			1	1	1	3	2	2	2	2	2	99	2	2	2	2	2	2				
2	2	2	1	1	1	2		1	1	1	3	2	1	2	2	2											
2	2	2	2	1	1	2		1	1	99	2	2	2	2	2	2											
2	2	2	2	1	1	2	1	2	1	1	3	2	2	2	2	2											
2	2	2	2	1	2		2	1	1	1	3	2	2	2	2	2											
2	2	2	2	1	1	2	1	1	1								99	3	3	2	2	2	2				
2	2	2	2	2	2			1	1	1	3	2	2	2	2	2											
2	2	2	2	1	2		1	2	1															1	4.9	3.8	2
2	2	2	1	2	1	2		1	1	1	6.3	3.1	2	2	2	2											
2	2	2	1	2	1	2		2	1	1	4.9	3.6	2	2	2	1	1	1.6	1.3	2	2	2	2				
2	2	2						1	1	1	1	1	2	2	2	2	1	1	1	2	2	2	2				
2	2	2	1	1	1	2	2	2	1	1	5.3	2.7	1	1	2	2											
2	2	2	2	2	2			2	1	1	6	4	2	1	2	2											
2	2	2	2	1	2		1	1	1								3	2	2	2	2	2	2				
2	2	2	2	1	2			1	1	2	6	4	2	2	2	2											
2	2	2	1	2	1	2		1	1	1	2	1	2	2	2	2	1	5	3	2	2	2	2				
2	2	2	2	1	1	2	1	1	1	1	1	1.5	2	2	2	2	99	0.5	0.5	2	2	2	2				
2	2	2	2	2	1	2		1	1								1	2	2	2	2	2	2				
2	2	2	2	2	2			1	1	2	2	1.5	2	2	2	2											
2	2	2	2	2	2			2	1															1	10.3	7.5	2
2	2	2	2	2	2			2	2								1	5.4	3.3	2	2	2	2				
2	2	2	2	1	1	2		1	1	1	1.5	1.5	2	2	2	2	1	0.5	0.5	2	2	2	2				

rafluct	radis	rasin	lano	lalsize	labsize	latend	lafluct	ladis	lasin	rino	rilsize	ribsize	ritend	rifluct	ridis	risin	lino	lilsize	libsize	litend	lifluct	lidis	lisin	hb	alc	esr	crp	cd4	gotoevent
																								11.7	1805	50	63.9		2
																								12.3	1210	52		378	1
																								12.4	2695	32	3.14	999	1
																								12.2		44	3.35	523	2
2	2	2																						9.8	1482	70	30.9		2
																								11.8	1587	39	16		1
																								13.2		27	46.7		1
																								9.6	664	50			1
																								15.7	946	42	34	181	2
																								10	2080	52	9.18	737	2
																								11.1	2231	8	3.14	732	2
																								7.7	768	69	76.3	528	2
																								14.2	3416	14	4.41	652	2
																								12.2				798	2
																								13.8	1824	17	3.14	662	2
																								12.9	1885	16	3.14	539	2
																								13	1430	10	3.14		2
																								11.3	1330	65	5.88	576	
																								13.2	2075	50	10.4	756	2
																								14.2	1584	13			2
																								13.9	988	40	31.3	282	2
																								12.9		32	7.32		2
																								15.9	2349	4	3.14	878	2
																								15.7	2460	4	3.14		2
																								12.4	2052	32	8.57	919	2
																								9.7	276	72	58	129	2
																								13.2	1349	37		365	2
																								12.1	1216			291	2
2	2	2	1	1	1	2	2	2	2															12.7	1460	38	11.9	719	2
																								14.1	3294	14	3.63	1416	2
																								11.1	996	52	19.1	528	2
																								9.9	2923	47	6.69	931	2
																								12.2	1440	12	3.17	494	2
																								10.8	1860				2
																								15.7	2754	16	12.9	1320	2
																								8.5	672				2
																								9.6	300	10	57	250	2
																								13					2
																								12.9	752	45	99		2
																								12.4	1900	20	3.17	806	2
																								12.8	228	14	3.14	619	2
																								11.7	3330	36	9.4	1441	2
																								12.9	2750	30	13.2	838	2
																								12.8	992	31	15.1	414	2
																								10.8	2088	66	45.1	989	2
																								11.5	1632	14	17.2	601	2
																								9	1125	92	45.8	458	2
																								12.3	2522	7	9.26	958	2
																								10.1	1375	54	7.59	451	2
																								12.6	1562	63	34.6		2
																								12.3	1881	14		802	2
																								9.3	1271	80	37.7		2
																								9.8	2070	84	26.9	505	2
																								12.1	1136	23	19.8	383	2
																								11.2		45	11.8		2
2	2	2																						15.4	1806	8	12.4	751	2
																								12.7	2233	45	12.4	537	2
																								10.8	1309	30	4.45	710	2
																								13.4	1170	42	8.98		2
																								11.7	1900	30	24.4	767	2
																								12.1	2622	37	3.02	1031	2
																								12.2		30	3.02	641	2
																								9.2	2832	10	3.14	1059	2
																								11.1	2438	45	19.2	905	2
																								10.3	2700	57	14.5	791	2
																								13.9	1624	19		699	2
																								11.9	1392	26	37.2	304	2
2	2	2																						13.9	1344	16	62.9	359	
																								12.7	1470	39	16.9	498	2
																								11.6	1488	44	3.02	805	2

rcnos2	rclsizes2	rcbsizes2	rctends2	rcflucts2	rcdiss2	rcsins2	lcnos2	lclsizes2	lcbsizes2	lctends2	lcflucts2	lcdiss2	lcsins2	ranos2	ralsizes2	rabsizes2	ratends2	raflucts2	radiss2	rasins2	lanos2	lalsizes2
1	1	1	2	2	2	2	1	3.2	3.1	2	1	2	2									
							1	5.5	4.5	1	1	1	1									
							2	3	4	1	2	2	2									
1	4	5	2	1	2	2																
1	4	4	1	2	1	1																
							1	8	6	2	1	1	1									

labsizes2	latends2	laflucts2	ladiss2	lasins2	rinos2	rilsizes2	ribsizes2	ritends2	riflucts2	ridiss2	risins2	linos2	lisizes2	libsizes2	litends2	liflucts2	lidiss2	lisins2	afbs2	cultures2	xperts2	rifrs2	sensits2	biopsys2	necrosis
																			2	2	2			1	1
																			2	2	1	2			
																			2	2	1	2		1	1
																			2	2	2			1	2
																			2	2	2			1	1
																			2	2	1	2		1	1

pilldayss	isoniazid	rifampicl	pyrazin1	ethambu	strepto1	vomits2	diarrs2	atts2	hbs2	alcs2	esrs2	crps2	cd4s2	fupevent	event	progres	dissem1	fluct	dischar	sinus	hbevent	alcevent	esrevent	crpevent	cd4even
															2										
															2										
															2										
															2										
														1	2										
0	300	450	1500	800		2	2	2	14	1659	3	3.17	362												
															1	1	2	1	1	1	12.1	1920	10	4.9	
0	300	450	1000	800		2	2	2	13	1995	27	26	1007												
															1	1	2	2	1	1			22	7.23	691
0						2	2	2	10.9	1488	78	25.9	745	2	1	1	2	2	2	1		1386	57	5.01	486
0	300	450	1500	800		2	2	2	10.3	2071	53	3.14	607	1											
															1	1	1	1	1	1	7.2	206	57		78
0	300	600	1500	1200		2	2	2	10.8	3132	24	15.7	1901												
														1											
														1											
														1											
														1											
														2											
														2											
														2											
														2											
0	300	450	1250	1000		2	2	2	11.8	1131	35	69.8	352												
														1											
														2											
														2											
														2	1	1	2	1	2	2	14.3	1984	5	3.02	
														2											
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eventsiz	breadth	cervical	axillary	inguinal	othsite	pilldaye	isoniazid	rifampici	pyrazin2	ethambu	strepto2	drugdos	follow	rebiopsy	rebiopsy	afbev	culturee	xpertev	rifrev	sensitev	biopsyev	necrosis	vomitev	diarrev	attev
3.5		1	2	2		0	300	450	1250	1000		1	1	2									2	2	2
4	4	1	2	2		0	300	600	1700	1400		1	1	2	3										
3	2	1	2	2			300	600				1		1		2	2	1	2		1	1	2	2	2
7		1	2	2			300	450	1000	800		1	1	1		1	1	1	2	1	1	1	2	2	2
		1	2	2		0	300	600	1500	1200		1		2									2	2	2

fup2mnt	progres2	dissem2	fluct2m	dischar2	sinus2m	hb2m	alc2m	esr2m	crp2m	fup6mnt	progres6	dissem6	fluct6m	dischar6	sinus6m	hb6m	alc6m	esr6m	crp6m	outside	cmc	totdur	surgtreat	surgproc	steriod	pentoxi	conserva	progress	dissemin	prtime
2	2	2	2	2	2			4	42	2	2	2	2	2	2	14.2	2079	2	15	0	6	6							
2	2	2	2	2	2					1										5	6	11							
2	2	2	2	2	2		2346	33	3.14	1											10	10							
2	2	2	2	2	2	12.9	1404	18	3.02	2										0									
2	2	2	2	2	2			8	3.14	2	2	2	2	2	2	12.4	1680	6	3.03	5	10	15	1	Left Limi	2	2	2	1	2	5
2	2	2	2	2	2			17	5.58	2	2	2	2	2	2			27	6.57		9	9			2	2	1	1	2	2
1																				8									
1										2	2	2	2	2	2	15.5	1792	4	3.03	0	10	10			2	2	1	1	2	2
2										2	2	2	2	2	2	11.6	1406	67	12.5	3	9	12	1	Left Limi	2	1	2	1	2	2
																				2									
																				3	15	18	1	Bilateral	1	1	2	1	1	4
2	2	2	2	2	2			7	5.21	2	2	2	2	2	2	10.6	2848	20	11.4	3	11	14			2	2	1	1	2	3
2	2	2	2	2	2	13.9				2											6								
2	2	2	2	2	2	11.6	1988	3	3.02	2	2	2	2	2	2	11.2	1850	3	3.16	0	6	6							
2	2	2	2	2	2		2432	18	5.25	2	2	2	2	2	2						6	6							
2	2	2	2	2	2	12.9	1435		3.02	2	2	2	2	2	2	13.1	1470	14	3.03		7	7							
2	2	2	2	2	2	11.4	1458	5	3.02	2	2	2	2	2	2	12.6	1564	3			7	7							
2	2	2	2	2	2	12.4	1666	34	3.14	2	2	2	2	2	2						9	9							
2	2	2	2	1	1			49	6.98	1											10	10							
2	2	2	2	2	2	11.6	11764	48	3.29	2	2	2	2	2	2														
2	2	2	2	2	2	14.4															9	9							
2	2	2	2	2	2			27	4.21	1											9	9							
2	2	2	2	2	2	16.2	2613	3	3.14	2	2	2	2	2	2		2535	4	3.02		7	7							
2	2	2	2	2	2	14.7	3105	6	3.02	1											7	7							
2	2	2	2	2	2					2	2	2	2	2	2						9	9							
2	2	2	2	2	2	12.3	1404	21	3.14	2	2	2	2	2	2						16	16	1	Left Limi	2	1	2	1	1	5
2	2	2	2	2	1	13.2			5.12	2										4	9	13							
2	2	2	2	2	2	14.4	2400	22	3.14	2	2	2	2	2	2	14.4	1892	18	5.47		9	9							
2										2																			2
2	2	2	2	2	2					2																			
2	2	2	2	2	2	13.1	2604	28	3.02	2											7								
2	2	2	2	2	2	11.1	2952	25	3.16	2	2	2	2	2	2			19	4.87		9								
2	2	2	2	2	2	10.4		22		2	2	2	2	2	2	14.7	1134	3	3.02	0	9	9							
2	2	2	2	2	2			17	3.22	2	2	2	2	2	2						10	10							
2	2	2	2	2	2	15	2592	17	11.3	2	2	2	2	2	2	15.3	3710	9	9.86	0	9	9							
2	2	2	2	2	2	10.2	1170	24	10.2	2										0	11	11							
2	2	2	2	2	2	7.8	702			2	2	2	2	2	2	11.6	1166	17		0	9	9							
2	2	2	2	2	2			34	16.5	1																			
2	2	2	2	2	2			5	3.14	2	2	2	2	2	2					0	9	9							
2	2	2	2	2	2					2	2	2	2	2	2	14.7	1925	7	3.02	0	8	8							
2	2	2	2	2	2	12.6	2262	6	3.02	1																			
2	2	2	2	2	2	10.7	2275	10	3.02	2																			
2	2	2	2	2	2	12.3	2047	25	9.67	2																			
1																													
2	2	2	2	2	2					2																			
2	2	2	2	2	2			13	4.92	2										1									
2																													
2	2	2	2	2	2	12.1	1760	8	6.13	2																			
2	2	2	2	2	2	13.5		17		2																			
2	2	2	2	2	2																								
1																													
2	2	2	2	2	2	9.4	1820	21	3.03	2																			
2	2	2	2	2	2	11.8	2310	45	5.66	2																			
1																													
2	2	2	2	2	2					2	2	2	2	2	2			38	8.17	0	9	9							
2	2	2	2	2	2	15.4				2	2	2	2	2	2					0	9	9							
2	2	2	1	2	2			40	12.5	2	2	2	2	2	2			61	15.5	10	12	22							
2	2	2	2	2	2	12.6	2400	18	4.41	2																			
2	2	2	2	2	2			42	14.5	2																			
2	2	1	2	2	2			35		2																			
2	2	2	2	2	2					2																			
2	2	2	2	2	2	12.5	1728	29	2.49	2																			
2	2	2	2	2	2			10	3.14	2	2	2	2	2	2	11.2	3081	1	3.03	0	6								
2	2	2	2	2	2					2																			
2	2	2	2	2	2	12.1	2961	22	18.9	2																			
2	2	2	2	2	2					2	2	2	2	2	2	13.3	1500	29	17	0	6	6							
2	2	2	2	2	2	13.2	1848	16	3.03	2																			